Atlantic Whitefish (*Coregonus huntsmani*) Culture Handbook

J. Whitelaw, J. Manríquez-Hernández, J. Duston, S.F. O'Neil and R.G. Bradford

Fisheries and Oceans Canada
Science Branch, Maritimes Region
1 Challenger Drive
Dartmouth, Nova Scotia
B2Y 4A2

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J. Whitelaw, J. Manríquez-Hernández[1], J. Duston[1], S.F. O'Neil and R.G. Bradford

Fisheries and Oceans Canada
Science Branch, Maritimes Region
1 Challenger Drive
Dartmouth, Nova Scotia
B2Y 4A2

[1]Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University, 58 River Road, Truro, NS, B2N 5E3

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ABSTRACT

Whitelaw, J., Manríquez-Hernández, J., Duston, J., O'Neil, S.F. and Bradford, R.G. 2015. Atlantic Whitefish (*Coregonus huntsmani*) culture handbook. Can. Manuscr. Rep. Fish. Aquat. Sci. 3074: vii + 55 p.

The Atlantic Whitefish (*Coregonus huntsmani*) is an anadromous species belonging to the subfamily Coregoninae, family Salmonidae. Atlantic Whitefish were designated 'Endangered' by the Committee on the Status of Endangered Wildlife in Canada in 1983 and have been listed and protected as endangered under the Canada *Species at Risk Act* (*SARA*) since June 2003. Its natural habitat has been compromised over the past century by acidification of the rivers and the construction of dams. Currently, this species is restricted to one watershed, the Petite Rivière in Lunenburg County, Nova Scotia. From 2000 to 2012, at the Mersey Biodiversity Facility, DFO personnel developed the methods to complete the life cycle in captivity. This culminated in the re-stocking of >12,000 juveniles in the Petite Rivière, from 2007 to 2009. In addition, >4,000 juveniles and 7,000 larvae were introduced in Anderson Lake, Dartmouth, NS as a trial aiming to assess the potential to establish lake-resident populations from cultured stock. In 2013, the Mersey Biodiversity Facility was closed. This handbook describes the culture methods and associated activities conducted at Mersey between 2000 and 2012.

RESUMÉ

Le corégone de l'Atlantique (*Coregonus huntsmani*) est une espèce anadrome qui appartient à la sous-famille des corégoninés, de la famille des salmonidés. En 1983, le Comité sur la situation des espèces en péril au Canada a désigné le corégone de l'Atlantique comme étant « en voie de disparition ». De plus, depuis juin 2003, il est inscrit sur la liste des espèces menacées et est protégé en vertu de la *Loi sur !es espèces en péril* (*LEP*). Au cours du dernier siècle, son habitat naturel a été compromis par l'acidification des cours d'eau et la construction de barrages. À l'heure actuelle, cette espèce est restreinte à un bassin versant, la Petite Rivière, dans le comté de Lunenburg en Nouvelle-Écosse. De 2000 à 2012, au Centre de biodiversité de Mersey, le personnel du MPO a mis au point des méthodes pour la réalisation du cycle de vie de l'espèce en captivité. Cela a abouti à l'empoissonnement de plus de 12 000 juvéniles dans la Petite Rivière, entre 2007 et 2009. En outre, dans le cadre d'un essai visant à évaluer le potentiel d'établissement des populations résidentes à partir des stocks d'élevage, plus de 4 000 juvéniles et 7 000 larves ont été introduits dans le lac Anderson, à Dartmouth, en Nouvelle-Écosse. En 2013, le Centre de biodiversité de Mersey a été fermé. Le présent manuel décrit les méthodes de culture et les activités connexes effectuées à Mersey entre 2000 et 2012.

INTRODUCTION

The Atlantic Whitefish (*Coregonus huntsmani* Scott, 1987) (Fig. 1) is an anadromous species endemic to Nova Scotia, Canada (Scott 1967). Its distribution is currently restricted to the Petite Rivière watershed in Lunenburg County, which includes Minamkeak, Milipsigate and Hebb Lakes (Bradford et al. 2004; Fig. 2). Since 1901, the construction of dams has prevented adults migrating to the open sea (Edge and Gilhen 2001). This 'land-locking' together with the recent introduction of invasive Smallmouth Bass (*Micropterus dolomieu*) and Chain Pickerel (*Esox niger*) further threaten the Atlantic Whitefish with extinction. Elsewhere along the south shore of NS, acidification of the watersheds has been associated with its decline in status (Edge and Gilhen 2001).

Atlantic Whitefish were designated 'Endangered' by the Committee on the Status of Endangered Wildlife in Canada in 1983 (COSEWIC 2010) and have been listed and protected as endangered under the Canada *Species at Risk Act* (*SARA*) since June 2003 (DFO 2006).The overall goal of the recovery strategy (DFO 2006) developed for Atlantic Whitefish is "*Achieve stability in the current population of Atlantic Whitefish in Nova Scotia, reestablishment of the anadromous form, and expansion beyond its current range.*".

The ability for this species to establish self-sustaining populations in any water body they do not presently occupy is poorly understood. However, the present demographics of Atlantic Whitefish suggest the likelihood is low that range extension will occur through natural colonization of new habitat (DFO 2004). The stocking of eggs and/or fish, produced via the captive breeding of Atlantic Whitefish, into locations not currently occupied by the species is an option both to facilitate range extension and to develop anadromy (DFO 2004, 2006). Supportive rearing of Atlantic Whitefish to enable stocking has never been attempted.

From 2000 to 2012, DFO developed culture methods at the Mersey Biodiversity Facility (herein MBF), Milton, NS. The development of spawning and early rearing techniques culminated in a supply of test animals to support research (see Bradford et al. 2004a, 2010, Cook 2012; Cook et al. 2010; Hasselman et al. 2007, 2009; Hasselman and Bradford 2012), experimental releases of juvenile Atlantic Whitefish downstream of the (at the time) impassable Hebb Lake Dam on the Petite Rivière and trial releases within Anderson Lake, NS, to explore the feasibility of establishing additional lake-resident populations by stocking F1 fish (Bradford et al. 2015). In 2013 the MBF was closed.

The principal objectives of this manuscript are to 1) document and describe the culture techniques for Atlantic Whitefish developed by Department of Fisheries and Oceans (DFO) personnel and associates at the MBF, 2) complement these techniques with

information from related species and 3) identify gaps in the knowledge to identify future research.

BACKGROUND OF ATLANTIC WHITEFISH

Taxonomy and systematics

The name "Atlantic Whitefish" was coined by Leim and Scott (1966, p. 104-105) to describe a species present in Milipsigate Lake, Lunenburg County, NS and Tusket River, Yarmouth County, NS. It clearly belonged to the genus *Coregonus*, but differed from the better known Lake Whitefish (*Coregonus clupeaformis*), by having more scales along the lateral line (91 – 100 vs. 70 – 85), a terminal mouth (rather than inferior, Fig. 3) and the presence of teeth in adults (Leim and Scott 1966, p. 102). Other approaches used to differentiate Atlantic Whitefish from Lake Whitefish include meristic and morphometric comparisons (Edge 1987; Edge et al. 1991; Hasselman et al. 2009); electrophoresis (Edge 1987; Bernatchez et al. 1991); mitochondrial DNA analysis (Bernatchez et al. 1991); microsatellite loci analysis (Murray 2005; Cook 2012; Crête-Lafrenière et al. 2012); and ontogenetic development (Hasselman et al. 2007). Its name, *Coregonus canadensis* (Scott 1967, p. 26), was later changed to *Coregonus huntsmani* in honour of Dr. A.G.S. Huntsman, the first to recognize this species as being unique to Canada (exists only within the Province of Nova Scotia), and also to avoid possible confusion with another whitefish with 'canadensis' in its name (McAllister et al. 1985; Scott 1987). In 1978 a new common name was proposed by Legendre, "Acadian whitefish" appeared in several publications (e.g. Edge 1984, 1987; McAllister et al. 1985; Campbell 1987; Bernatchez et al.1991). Nomenclature issues were resolved in 1999 when all parties agreed "Atlantic Whitefish" be the only common name (Edge and Gilhen 2001).

Understanding the taxonomic relationship between the whitefish species can be valuable when attempting to optimize culture methods and conservation management. Elucidating whitefish systematics has been challenging because many of the traditional (phenetic) taxonomy characteristics such as body shape and size, growth rate and numbers of scales and gill rakers can vary considerably depending on environment (Piers 1927; Scott and Crossman 1973; Edge et al. 1991). In addition, the current genomic determination is hampered by parallel evolution, phenotypic plasticity, trophic polymorphism, hybridization and introgression within the Salmonidae family in general (Crête-Lafrenière et al. 2012).

Coregonus huntsmani belongs to the subfamily Coregoninae, one of the three subfamilies of the family Salmonidae. Coregoninae contains three genera: *Coregonus*, *Prosopium* and *Stenodus* with 78, six and two species respectively (Froese and Pauly 2013). However, controversy persists. Genomic studies have indicated that *Stenodus* should be merged with *Coregonus* generating a subgenus (Sajdak and Phillips 1997;

Crête-Lafrenière et al. 2012; Blanchet et al. 2013). The creation of subgenera in *Coregonus* is not new. Lindsey (1981) proposed two subgenera based on feeding preferences and gill raker size: *Leucichthys*, plankton feeding ciscoes and *Coregonus*, benthic feeding true whitefish. However, this classification has problems, Atlantic Whitefish exhibit characteristics of both subgenera, and the phylogenetic relationship between the ciscoes and whitefish species do not follow this trophic division, both subgenera overlap (Edge 1987; Sajdak and Phillips 1997). Recent studies analyzing both mitochondrial and nuclear DNA indicate Atlantic Whitefish is a sister species to the rest of *Coregonus*, a branch which diverged *ca.* 15 million years ago (Crête-Lafrenière et al. 2012). In the same way, the Lake Whitefish complex (McPhail and Lindsey 1970) from North America were divided into four races from East to West to reflect their glacial refugial affiliations, i.e., Acadian, Atlantic, Mississippian and Beringian (Bernatchez and Dodson 1994; Bernatchez et al. 1999). The Beringian race is closer to the European whitefish (*C. lavaretus*) than the races of Lake Whitefish from the same continent (Bodaly et al. 1991; Bernatchez and Dodson 1994; Sajdak and Phillips 1997).

Whitefish culture methods to support harvest fisheries for the Eurasian species within the "lavaretus-clupeaformis" complex are better documented than for North American species. Commercial production in 2011 reached 3,958 tonnes, 67% of which were produced in the Russian Federation (FAO 2014). In North America, Lake Whitefish has been cultured since 1877 with the progeny of donor stocks originating from within the Great Lakes Basin distributed into many of Canada's inland waterways (Bradford and Mahaney 2004). More recently Lake Whitefish culture has been used to assist with the recovery of natural populations (Lasenby et al. 2001; Hooper 2006). Despite the early divergence of Atlantic Whitefish from the other whitefish, the rearing techniques developed for "lavaretus-clupeaformis" complex have been incorporated in this handbook, but their application needs to be validated.

Geographic distribution

The historical geographic distribution of the Atlantic Whitefish is unclear because species were misidentified. Piers (1927) suggested the three specimens captured in May 1923 and 1924 in Milipsigate Lake, Lunenburg County, were the first record of *Coregonus* in Nova Scotia. However, the author did not consider the two specimens captured in September 1919 in the mouth of the Sissiboo River, Digby County (Huntsman 1921). The specimens from Sissiboo River were initially identified as *Coregonus quadrilateralis* (currently *Prosopium cylindraceum*) and only posteriorly suspected to be Atlantic Whitefish because they were captured in brackish water (Bigelow 1963). The first certain record of Atlantic Whitefish corresponds to the three specimens described by Piers (1927). Although they were originally determined as a variant of *Coregonus labradoricus* (currently *C. clupeaformis*) there is a clear description that confirms they were Atlantic Whitefish. The holotype for Atlantic Whitefish was a

specimen from the Tusket River caught in November 1954 (Scott 1987). The Tusket River population was extirpated around 1982 (Scott and Crossman 1973; Bradford et al. 2004). In the 1950's specimens suspected to be members from this anadromous population were caught from Wedgeport to Halls Harbour (Leim and Day 1959). That they were Atlantic Whitefish was confirmed by meristic and morphometric analysis (Edge 1987) the same as specimens captured in Milipsigate Lake. Atlantic Whitefish were reported in the LaHave River in 1938 (McKenzie 1940) and 1997 (Edge and Gilhen 2001), but none were caught in 2004 (Bradford et al. 2004). However, they were present in Minamkeak and Hebb Lakes, in the same watershed as Milipsigate Lake which drains into Green Bay, 10 Km SW from the LaHave River (Edge 1984; Edge and Gilhen 2001; Bradford et al. 2004).

Habitat and basic biology

Two Atlantic Whitefish populations are recognized: one (extirpated) anadromous in the Tusket River watershed, and one landlocked in the Petite Rivière watershed (Edge 1984). Differences in their habitat and trophic niche are reflected in differences in their morphology (Edge et al. 1991). However, some of the morphological differences could be attributed to differences in the collection date and time preserved since changes in the ecosystem can change the population size distribution (Haedrich and Barnes 1997; Milazzo et al. 2005). Adults of the anadromous population once migrated up the Tusket River from mid-September to early November. They were thought to have overwintered in the estuary and returned to the sea between February and April (Edge 1984). Spawning was thought to occur during fall or early winter. Two females captured Nov. 4, 1967 and October 11, 1982 both had well developed ovaries but were not ready to spawn (Edge 1987). Pearl organs were visible on adult males captured in the Annis River, a branch of the Tusket River, in October 1982 (Edge 1984). These white bumps appear on the head of the males during the spawning season; their distribution and number differ between Atlantic Whitefish and Lake Whitefish (Edge et al. 1991; Fig. 3). The gonads of fish in the Petite Rivière in November 1982 at 10 °C were well developed, but were not fully mature. By comparison, specimens captured in May 1983 were in a post-spawned state with small gonads (Edge 1987). Males from Hebb Lake captured in November 1982 possessed pearl organs, similar to the observations from Tusket River fish (Edge 1984). Body size at age of first sexual maturity is unclear, all the specimens larger than 17.8 cm standard length (SL) were mature; one specimen of 14.8 cm SL was immature (Edge 1987). Only one juvenile has been captured, a young-of- the-year fish, 8.4 cm total length, from along the shore of Hebb Lake in June 2000 (Hasselman et al. 2005).

Atlantic Whitefish are opportunistic feeders. The stomach contents of the anadromous form captured in seawater included amphipods, decapods, periwinkles (*Littorina littorea*), other marine invertebrates, and a few blades of eelgrass (*Zostera marina*; Leim

and Scott 1966; Scott and Scott 1988). By comparison, the landlocked population ate Cladocera, blackfly larvae, flying ants (Hymenoptera), Plecotptera, Hemiptera,Coleoptera, fish and unidentified insect larvae (Edge 1987). The prevalence of insects in the diet in Atlantic Whitefish is another difference from Lake Whitefish, the latter preferring benthic organisms (Edge 1987). These trophic differences conform to morphological differences in mouth shape and position, also in their apparent depth preferences. Atlantic Whitefish were more abundant from the surface to 11 m depth, while Lake Whitefish preferred deeper water (Edge 1984, 1987).

Minamkeak, Milipsigate and Hebb Lakes are shallow (13 to 16 m; Edge 1987). The lakes stratify during the summer, but at least in Hebb Lake a cold water hypolimnion is not present. Only in June 1983 was a gradient detected between the surface (26 °C) and the bottom (14 °C), but in May, August and September there was no stratification (Edge 1987). Consequently, extensive volumes of cooler water may not always be available to Atlantic Whitefish during the summer. Hence these lakes may provide only limited suitable habitat for Atlantic Whitefish, which perhaps explains why this species contributed less than 5 % of the ichthyofauna captured in the three lakes, total 751 fish (Edge 1987). pH varies among the three lakes from 6.0 and 4.5 and is considered to be generally high compared with other watersheds along the south shore of Nova Scotia (Watt et al. 1983; Underwood and Schwartz 1990), where acidification has seriously impacted aquatic life, the most high-profile being the Atlantic Salmon (*Salmo salar*, Edge 1984).

WATER QUALITY

Mersey Biodiversity Facility

Mersey Biodiversity Facility (Mersey BF) was a Federal Government hatchery, located on the lower Mersey River in Milton, Queens County, NS. From its construction in 1968 until 1974, the main species cultured were Brook Trout (*Salvelinus fontinalis*). Since 1974 its principal role was the production and enhancement of Atlantic Salmon (Goff and Forsyth 1979). Atlantic Whitefish were reared there from 2000 to 2012. The infrastructure consisted of a hatchery building and 36 exterior square concrete Swede ponds (20 at 7.6 m wide, 16 at 11 m wide, Fig. 4). While the majority of the Atlantic Whitefish work occurred in the hatchery building, post-larval fish and juveniles were largely reared in the Swede ponds. The facility was gravity-fed from the Mersey River, through both surface (3 m) and deep (9 m) water intakes above a dam (MacDonald and Ratelle 2011). To counter high summer surface water temperatures up to 25 °C, water sources were mixed and oxygen injected into the water. In winter, the intake water was 0.0 to 0.3 °C (Fig. 5).

The water quality of Mersey River has been classified as 'poor' for aquatic life (Khan et al. 2003). The authors evaluated a data series from 1972 to 2000 and determined that

pH, aluminum, lead, nitrate and iron concentrations did not meet the Canadian Water Quality Index (CWQI). Mersey River also failed the standards set by the European Inland Fisheries Advisory Commission (Dennis and Clair 2012). Both pH and aluminum concentrations were outside the standard, with a mean pH of 5.1 and 0.04 mg/L of ionic aluminium. The inflow water to the Mersey BF sampled on October 2009 and December 2010 had a pH of 5.86 and 5.09 respectively; total aluminum was 1.4 and 0.2 mg/L, respectively; lead and nitrate were lower than their detection limit, 0.5 µg/L Pb and 0.05 mg/L $N\text{-}NO_2$, respectively; total iron was 0.26 and 0.34 mg/L, respectively (Appendices I and II). However, these two sampling events are inadequate to determine the water quality due to the large seasonal and inter-annual variation. For example, pH was lowest in winter, then increased during spring and summer, then declined in the Fall (Clair 1995). Factors contributing to this variability include rainfall, groundwater, snowmelt or runoff; and the time of interaction between the water and the bedrock (Underwood and Schwartz 1989; Clair 1995). Over 25 years the CWQI of the Mersey River has fluctuated greatly, ranging from 20 to 75, but only in 1975 the index was higher than 40, the upper limit of the "poor" condition for aquatic life (Khan et al. 2003). In addition to the parameters above, total barium in the Mersey inflow in December 2010 exceeded the standard recommended for freshwater aquatic life (Appendix II; Nagpal et al. 2006). To improve the water quality for fish culture, the inflow water was treated with calcitic ($CaCO_3$) limestone gravel. To maintain its performance, the limestone gravel was cleaned weekly, and changed at least every two months. The treatment increased the pH to 6.43 in 2009 and to 5.45 in 2010; and the hardness of the water from 3 to 6 mg/L in 2009 and 5 mg/L in 2010, but total alkalinity remained undetectable (<5 mg/L; Appendix I). All the heavy metals noted above increased slightly but as pH and hardness also increased, their potential toxicity decreased.

Despite the less than ideal water quality, Mersey BF successfully produced large numbers of brook trout, Atlantic salmon and latterly, Atlantic Whitefish. The low pH level even had one beneficial property, its disinfectant properties helped to keep fish free of pathogenic diseases (Cotter and Hill 2003). In addition, the high tannin content may have been responsible for the lack of stickiness of the Atlantic Whitefish eggs reared at Mersey. Tannin is used in the culture of other species to remove the adhesive from sticky eggs (Bokor et al. 2013). The disparity between the pessimistic water quality indices and the excellent fish production record at Mersey highlights the difficulties determining what chemical species are toxic to aquatic life. The interactions between chemicals, the species of fish and life history stages add to the complexity. The toxicity of aluminum increases with temperatures and also with zinc and copper concentration (Hutchinson and Sprague 1989; Grensemer and Playle 1999). By contrast, the toxicity of aluminum is reduced by total organic carbon, humic acids, iron, calcium, fluoride, and silicon concentration (Peuranen et al. 2004; Grensemer and

Playle 1999; Dennis and Clair 2012). For example, the eggs of European whitefish were more resistant to low pH than larvae (Keinänen et al 2003). Atlantic Whitefish seemed to be more resistant to low pH that other whitefish species, however the interaction with aluminum was not considered (Cook 2012). In addition, among European whitefish species chronic exposure to sub-lethal pH and aluminum concentrations affected gonadal development, reduced plasma sodium and chloride concentrations, increased blood glucose concentration, reduced somatic growth rate and caused gill damage (Vuorinen et al. 1990; Vuorinen and Vuorinen 1991; Rask et al. 1992). The water quality at Mersey may be a factor contributing to the observed low gamete production of Atlantic Whitefish in comparison with Lake Whitefish in Ontario.

Petite Rivière watershed

The natural habitat for Atlantic Whitefish, three lakes in the Petite Rivière watershed, is located *ca.* 35 km NE from Mersey, therefore the surface water temperatures are similar, peaking at 25 ºC (Cross 2012). pH of the Petite Rivière system is higher than most other watersheds along the south shore of Nova Scotia due to the local geology. pH in Milipsigate and Hebb Lakes in summer is typically >6, and in Minamkeak Lake >5.5 (Anonymous 2010; Page 2010; Cross 2011). However, in December and January the pH decreases to 5.5 or lower (Cross 2012). pH 4.5 is critical for Atlantic Whitefish eggs, and early larvae survival and may be a limiting factor to recovery efforts. At pH 4.5, post fertilization egg survival was 22 %; and 20 % hatched, and among the resultant larvae, 45% survived (Cook 2012). pH surveys appear to have been restricted to water samples taken from the shore (Edge 1987; Cross 2011, 2012). pH data from the bottom of the lake in winter is needed, where eggs likely incubate. Special attention should be given to Minamkeak Lake since surveys conducted between 2009 and 2012 indicated this lake is the least acidic of the three lakes (Anonymous 2010; Page 2010; Cross 2011, 2012). Conversely, historically and in surveys conducted in 2004 and 2005, Minamkeak Lake had the highest pH, mean 7.4 (Ginn et al. 2008).

Apart from pH, the water chemistry of Petite Rivière watershed and Mersey are similar. Low alkalinity, low conductivity and presence of tannin are common characteristics. Alkalinity in the three lakes was usually below 1.0 mg/L of $CaCO_3$ with a peak of 3.0 mg/L in Hebb Lake in summer 1982. In the winter, the values were under the detection limit of the technique used (Edge 1987). Conductivity was low, 20 µS/cm, with a peak in winter only in Hebb Lake, 65 µS/cm (Edge 1987). Colour was low in summer, and a little higher in winter, 50 to 100 hazen units. These values were lower than nearby rivers (up to 400 hazen units), indicating lower tannin concentrations (Edge 1987). Surveys conducted between June and July 2009, June and September 2010, and May and September 2011, indicated the three lakes had similar average conditions: temperature 21.7 ºC, conductivity 29 µS/cm, total dissolved solids 0.026 g/L, salinity

0.01, oxygen saturation 87.9 %, and pH 6.25 (Anonymous 2010; Page 2010; Cross 2011).

BROODSTOCK

Wild fish collection and acclimation to captivity

Collection of adult Atlantic Whitefish is strictly regulated by DFO. License applications should be submitted well in advance of any planned activities. Atlantic Whitefish are susceptible to stress from handling and every effort must be made to minimize this stressor. Mortality post-capture was not uncommon at Mersey. A good location to capture adults was the pool below the dam between Hebb and Milipsigate Lakes (Fig. 6). In addition, in 2003 a few were caught with a trap net in Milipsigate Lake. Various collection methods were tried: seining, angling, trap netting, limited gillnetting and electrofishing. In the pool, the best method was fly-fishing with barbless hooks, since it minimized abrasion of the skin associated with netting. To facilitate capture, a low water flow below the dam was important. Bridgewater Water Supply can control the discharge rate if required. However, the dam in place during the period the collections (2000 – 2004) has since been replaced with a different structure. Capture attempts were performed only when water temperature was <16.5 °C, usually in the fall or spring. High temperatures greatly increase the risk of mortalities. The skin of Atlantic Whitefish is delicate and scales are easily lost by abrasion. Fish were moved in water from their point of capture to the holding tank used during their transport to MBF whenever feasible, and all nets were of a fine knotless mesh. In a normal collection day, only 1 or 2 fish were caught (Table 1). The record was 12 fish in one day. Due to the low capture rate, it was important to start the collection season as early as possible, to allow collections to take place within the acceptable temperature window.

The trucking water was from the same source as the fish, plus 0.2 % non-ionized salt (NaCl). Products such as API Stress Coat® or Syndel Vidalife® can help reduce trucking stress, and the former was used sometimes when Atlantic Whitefish were being trucked. Oxygen concentration during trucking was maintained at 90 - 100 % saturation and never dropped below 60 %. Depending on the tank volume and number of fish, it was sometimes unnecessary to add oxygen. The trucking tank should be as large as possible with round corners to reduce the incidence of nose damage. Both the trucking water and receiving water were <16.5 °C. When the truck with the fish arrived at MBF, prior to transfer of the newly captured fish to the holding tank, Mersey River water was pumped slowly into the tank on the truck. This helped acclimate the fish to the slight differences in water chemistry between the lakes and Mersey River. Newly captured fish were transferred to a holding tank and held for 24 hours in darkness. The tank was about 1 m deep and covered with a tarp. This midsize tank made it easier to visually monitor the fish. The following day a prophylactic salt treatment was normal practice.

Sufficient NaCl (*ca.* 20 g/L) was dissolved in a bucket to elevate the salinity in the holding tank to 20 ppt. The inlet water was turned off, and the brine was broadcast over the tank surface and gently mixed. After 45 minutes the freshwater supply was turned back on to dilute the salt away. Salt baths were performed routinely after any significant disturbance or if the fish showed signs of wounds or fungus.

Beginning three days post-capture, the fish were offered a 100 % krill (*Euphausia pacifica;* source: http://www.fishalicious.ca) diet about three times per day for about three weeks. The freeze dried krill was broken up into bite-size pieces either by hand or by a small handheld coffee grinder. As the feed response improved the fish were gradually weaned off the krill and onto a dry pellet (floating pellet #3, Corey Aquafeeds, NB; 42 % protein, 14 % fat). The water inflow rate was slow initially but increased once the fish started feeding to help maintain tank hygiene. A water flow of 38 to 57 L/min was suitable for the holding tank (*ca.* 4000 L; 2.4 m diameter, 0.9 m water depth). As the fish became acclimated to the rearing tank, the tarp was gradually pulled back to allow in light, but the tank always remained partially covered. Recently captured fish were very sensitive to abrupt human disturbance and changes in light intensity. They typically exhibited a frantic escape response which resulted in them banging their noses against the tank wall, causing tissue damage and fungal infections which sometimes led to death. Hence visual inspections needed to be conducted regularly but with great care, and any signs of fungus treated with a salt bath. During the first few years of supportive rearing activities swim bladder over-inflation occurred during the first week in up to 10 % of the fish. The cause was unknown. The fish swam around "belly-up" and did not recover, and had to be euthanized. Puncturing the swim-bladder with a needle was not attempted.

In the first year, fish were transferred in water to a relatively deep holding tank (1.8 m depth) about a week after they were fully weaned onto a pelleted diet. However, this deep tank proved unsatisfactory because it was impossible to properly observe the fish due to the high tannin content discoloring the water. Under conditions where the water is clear, deep tanks are recommended for Atlantic Whitefish. At Mersey, the shallow tanks (1 m depth) facilitated early detection of fungus, which could be quickly treated with a salt bath. However, at low temperatures <5 °C, salt baths were less effective. Handling was kept to a minimum, and never in summer when temperature exceeded 16 °C. Fish were measured no more than three times per year (spring, fall and winter). If handling during summer it was essential the rearing water was cooled to <16.5 °C by setting up a semi-recirculation system that included a chiller and supplemental oxygenation. Water exchange was at least 1/3 tank volume per day to maintain tank hygiene. Fish tolerated low water velocity, but a higher flow rate improved both their swimming behaviour and the water quality.

Fork length of Atlantic Whitefish captured from the lakes 2000 to 2003 ranged from 20 to 34 cm, with a mean of 24 cm. By comparison, fish caught from the Tusket River in the last century were up to 40 cm FL. The lakes appear to be food-limited, preventing the fish from expressing their growth potential. The rapid growth of captive fish at Mersey supports this hypothesis. European whitefish (*Coregonus lavaretus*) in captivity also grew faster and bigger than wild fish (Szczepkowski et al. 2010). Over-feeding and obesity became a problem at Mersey in the early years. A commercial salmon diet with 20 % fat proved unsuitable, resulting in excessive fat deposits around the organs in the body cavity, but not between the myotomes (Fig. 7). A trout diet with 14 % fat (Ewos Vita) resolved the obesity problem. Atlantic Whitefish are very active and swim constantly against the current in the mid water column. When offered food they usually ingest the pellets in the water column, and very rarely off the tank floor. Because cultured fish grew faster than wild fish, it was not possible to estimate the age of the wild fish captured. Moreover, there is no ageing reference material to read the scale rings, so it was not clear if Atlantic Whitefish had annual rings or two rings per year, summer growth and winter growth (DFO 2010).

Unhealthy fish typically swam close to the surface and had 'discoloured' skin. The skin colour of unhealthy fish was usually relatively pale, but sometimes it was darker than normal. Mortality during the first year in captivity at Mersey was up to 60 % (Table 2). Mortality was often associated with stress due to handling during spawning season, high temperature or disturbance. Necropsies usually failed to link death with a pathogenic disease. More commonly, death was associated with fungus (*Saprolegnia* sp.). Pectoral and pelvic fins were prone to fungal infection at Mersey, and severe attacks caused complete fin loss. European whitefish also suffer mortality due to the stress of handling and spawning. Mortality from age of 1+ to age of 4+ was 20 % for males and 40 % for females (Szczepkowski et al. 2010).

The genetic diversity of Atlantic Whitefish in the three lakes is very low, severely restricting the scope of potential breeding and restocking initiatives (Murray 2005). Consequently, F1 fish produced at Mersey have not been released into Petite Rivière's lakes. Releases have been restricted to either the river below the first dam or Anderson Lake near Dartmouth (Bradford et al. 2010). By comparison, in Ontario, there is no breeding program for Lake Whitefish. Wild adults are captured in Simcoe Lake by trap-net and stripped *in situ*, then fertilized eggs are transferred to the hatchery 300 km away (Harris and Hulsman 1991; Hooper 2006). In Europe, there is some experience of maintaining broodstock in captivity (Gillet 1991; Szczepkowski et al. 2010).

Sexual maturation

Spawning among Atlantic Whitefish at Mersey typically began in early December and could extend to mid-January. Some years spawning started in mid-November. Feeding of the adults selected as potential spawners ceased in the first week of November.

Post-spawning it was important to get fish back onto feed as soon as possible since delaying re-feeding has associated with deterioration in appetite. Usually, fish took three to four weeks to eat normally again. As spawning season progressed, the incidence of overripe eggs and deterioration of milt increased. Both problems were documented on January 18, 2005 and January 5, 2006. Determining the sex of immature Atlantic Whitefish is not possible using external characteristics. By contrast, between November and January, maturing males can be identified by pearl organs (also called nuptial tubercles) around the opercula and head region (Fig. 3a). Females have no physical characteristics signaling of sexual maturation aside from the swelling of the abdomen due to ovary development and a slight protrusion of the vent. However, some females produced eggs with no obvious swelling of the abdomen.

Fish captured in May can mature successfully in their first winter in captivity. Egg quality was usually good, but acquiring sufficient good milt was a chronic problem. The timing of completion of sexual maturation of each sex is not always synchronized. Injections of Ovaprim™, (Syndel, analogue of salmon GnRH), both in the body cavity and intramuscular (23 gauge needle), failed to improve milt production during winter 2003-04 and was associated in the death of 8 of 10 males within a month. Larger needles caused wounds in the fish and smaller needles hindered the release of the Ovaprim.

Age at first sexual maturity among wild fish is unclear. Among F1 fish, some males matured age 1+, and by age 2+ both females and males have produced gametes. Fecundity increases with body size (Fig. 8). Females 25 cm fork length (FL) produce 1,000 to 2,000 eggs, and a 47 cm FL fish produced 12,500 eggs. Fecundity of captive wild and F1 females was similar (Bradford et al. 2010; Fig. 8). Eggs diameter is 2 - 3 mm (Hasselman 2003). Among fish reared at Mersey, egg quality decreased among fish older than 5 years. The life span in captivity at Mersey was usually 7 to 8 years, maximum 9 years. European whitefish show similar traits: F1 males matured age 1+, and females age 2+, with fecundity and egg quality higher among fish age 3+ and 4+, no older fish were evaluated (Szczepkowski et al. 2010). Among European whitefish, the timing of sexual maturation has been delayed by up to two months using a long day photoperiod from fall through December (Gillet 1991). We suggest photoperiod manipulation may be useful to synchronize maturation between male and female Atlantic Whitefish.

Two methods for spawning were tried at Mersey; natural spawning where fish spontaneously released their gametes in the tank, and hand stripping. Natural spawning became the preferred method at Mersey because it resulted in a higher hatch percent, presumably due to a higher fertilization rate resulting from a lower incidence of over-ripening.

Natural spawning

In November, broodstock were either transferred to a spawning tank or the holding tank drain was modified to retain the fertilized eggs. Spawning tanks were equipped with a catchment device at the outflow to allow daily observation of any eggs released into the tank. Six males and six females were placed in a spawning tank. Among these, five or six females typically produced good eggs. Natural spawning normally occurred at night. The specific gravity of the eggs is greater than water, so fertilized eggs sank to the bottom of the tank and were collected with a dip net or from the catchment device. Natural spawning normally occurred in early December (2.0 °C in 2005, 3.5 °C 2007), but also occurred in late-November (6.6 °C in 2005, 8.0 °C in 2006) and in early January (0.9 °C in 2006, 1.9 °C in 2007).

Artificial spawning

Spawning and fertilization at Mersey was conducted in a cold and dry room, 10 °C or less. Since whitefish are pagophilic (preference for water ice, Cherniaev 2013), the plastic containers used to receive the gametes were placed in an ice bath or chilled water bath. Broodstock were anesthetized using Tricaine methanesulfonate (MS222, about 0.15 g/L), rinsed in freshwater to remove the MS222, then damp dried to avoid water getting on the gametes. Due to the low production of milt by Atlantic Whitefish males, they were stripped first. Then, if enough milt was collected, females were stripped. Stripping the males required two people; one to hold the fish and extract the milt, another to collect the milt from the vent with a medicine dropper (or eye-dropper). Eggs were stripped directly into a dry plastic bowl (Fig. 9a). To extrude all the eggs, the manual stripping action needed to be repeated about ten times. Stripping out all the eggs reduced problems the next spawning season due to egg shells and debris. The rate of over-ripening of Atlantic Whitefish eggs in the body cavity is not known. To minimize the risk of over-ripening, females were checked for ovulation twice a week. A salt bath (20 ppt per 45 min) was conducted once a week to prevent fungus following the handling. Among European whitefish eggs, over-ripening was evident 3 - 4 days post-ovulation at 5 °C and 1 - 2 days at 7.5 to 11 °C (Gillet 1991).

Cryopreservation and successful thawing of milt from Atlantic Whitefish held at Mersey has been achieved, but results have been variable (de Mestral Bezanson et al. 2010). Milt was mixed immediately in a 1:3 ratio with cryoprotectant (0.3 M glucose, 20 % glycerol, and 80 % distilled water). Milt from one male was often insufficient to fill a 0.5-ml straw, so milt from several males was combined. The mixture of milt and cryoprotectant in the straw included a 1 cm air space to allow for expansion during freezing. Details of the freezing and thawing protocol are described in de Mestral Bezanson et al. (2010).

The dry method of fertilization was used at Mersey. At least a 3:1 male:female ratio was usually employed, but as high as 10:1 ratio in some instances (Table 3). 1:1 ratio has been used but was deemed unsuitable because of the low genetic variability in the wild population. Typically, the eggs from a single female were fertilized with milt from 3 males as follows: eggs were sub-divided into three containers, and each batch fertilized with the milt from a single male. Eggs and milt were mixed thoroughly and left for two minutes (Fig. 4.4 b and c). Freshwater, pH >5.5 was then added, and let stand for two minutes. Sperm were motile longer in freshwater than in 15 or 30 ppt salinity, 32 s vs. 1.72 and 1.52 s, respectively (Cook and Bentzen 2009). Eggs were gently stirred occasionally to avoid clumping, then rinsed and allowed to water harden in their incubations units. After one to two hours, when water hardening was complete, water flows in units was increased if egg movement was desired. Fresh eggs released from the female are not adhesive, but become more adhesive as they water harden. However, sticky eggs were not observed at Mersey, likely because of the tannin in the water. Eggs incubated at the Aquatron (Dalhousie University, Halifax, NS) in tap water were sticky (A. Cook pers. comm. Dec. 2013).

Hand stripping was very stressful, particularly for the females, and scale loss could be heavy unless great care was taken. To reduce skin damage it was essential the hands of persons holding the fish were wet and gloveless. After stripping, swim bladder over-inflation occurred in some fish, the same phenomenon as occurred post-capture.

Egg Incubation

Fertilization to hatch at Mersey took about three months at temperatures ranging from 0 to 4 °C. Hatching started at *ca.* 120 - 130 degree-days (Hasselman 2003). Eggs were incubated either in the dark or dim light.

Incubation methods and health risks

A variety of incubation units were tried at Mersey: 6-litre MacDonald jars, 3-litre MacDonald jars (a normal jar cut in half; Fig. 10a), Heath trays (Fig. 10b), 2 feet circular tanks and upwelling tanks. Each unit had advantages and disadvantages. The problem with 6-litre MacDonald jars was removing dead eggs before fungus appeared because the dead eggs were mixed up with the live eggs. By contrast, dead eggs of Lake Whitefish conveniently float to the top of the jars and can be easily siphoned out (R. Zheng pers. comm.). Use of 3-litre MacDonald jars made it easier to pick out dead eggs, and also use a lower stocking density, no more than 2,000 eggs. The water flow to the 3-litre MacDonald jars was between 2 and 3 L/min. By comparison, Lake Whitefish eggs are successfully incubated in a 6-litre MacDonald jar containing 70,000 eggs (Hooper 2006; Fig. 10c). In Heath trays, eggs were incubated in a monolayer. The best incubation method was to set up the Heath trays in fibreglass flow-through

salmon egg troughs. Twelve Heath trays each with 2,000 eggs were set up in each trough. The water flow was 12 L/min.

Egg survival was highly variable. The principal problem was fungus. Chemical treatment of the eggs was not attempted until 2004. To prevent fungus, prophylactic salt baths (20 ppt) were performed twice a week. If fungus was visible, eggs were given a 50 ppt salt bath for 1 h (41.61 g NaCl per litre). This high concentration of salt slowed the rate of fungus growth, but did not eradicate the problem. Formalin treatment (1:500 per 15 min) of Atlantic Whitefish eggs was tried at Mersey but was not effective. Picking dead eggs required special small forceps or wire loop pickers (wire with small circle at the ends). Following an initial pick after water hardening, dead eggs were not picked during the first three weeks of incubation; hence high mortality could not be attributed to physical disturbance from picking activity. After three weeks incubation, dead eggs were picked every two days when mortality was high, and 'as needed' during the rest of the incubation period.

High mortality was consistently experienced at roughly day 14 post-fertilization, early gastrulation stage at *ca.* 14 degree days. The reason for this heavy loss is unknown. Egg survival was generally higher when fish spawned naturally in a tank compared to hand stripping, suggesting over-ripening may have compromised egg quality. The challenge was to keep eggs alive to the eyed stage. From eying onwards the eggs and larvae were very hardy. Among Lake Whitefish eggs cultured in Manitoba and Quebec, the mortality rate was 50 - 60 %, similar to the experience at Mersey. However, losses at MBF were sometimes much higher. In spawning season 2005/06, the first year F1 fish were used as broodstock, in six of nine crosses almost all the eggs died. Among European whitefish species, the survival rate to the eyed-egg stage varied with the age of broodstock, 0 % at age 1+, 59 % at age 2+, 62 % at 3+ and 64 % at 4+ (Szczepkowski et al. 2010).

The egg incubators at Mersey received ambient surface water that ranged from 0.14 °C in December to 10.40 °C at the end of April (Spawning season 2002/03). Accumulated degree-days increased gradually until the end of March, then increased more quickly following 'ice-out' (Fig. 11). The water supply was treated with limestone gravel to increase the pH prior to entering the hatchery.

Stages of development

Atlantic Whitefish eggs from wild fish collected in spring 2000 and spawning in winter 2000 were yellow to amber in colour, spherical in shape, demersal and slightly adhesive (Hasselman 2003; Hasselman et al. 2007). The chorion was colourless, unornamented and smooth. Eggs contained a single large oil globule surrounded by smaller oil globules of variable size either scattered or in clusters of three to six. Eggs fixed in formalin had a diameter of 2.7 $\pm$ 0.0 mm (mean $\pm$ SD) and a volume of 10.30 µL

Unfertilized eggs (water hardened for 29 h) were 4.01 ± 0.85 mm diameter with a volume of 38.06 ± 21.68 µL (Hasselman 2003; Hasselman et al. 2007).

Stages of development of Atlantic Whitefish eggs (Hasselman et al. 2007):

- Zygote. At the time of fertilization. Telolecithal, with the blastodisc concentrated at the animal pole, and unsegmented yolk at the vegetal pole, the yolk had a diameter of 3.85 ± 0.86 mm, water hardened egg diameter of 4.01 ± 0.85 mm.
- Discoblastula. 2.5 h post-fertilization. Egg exhibits meroblastic cleavage, blastomeres formed by discoidal cell division (Fig. 12a). Oil globules were concentrated below, but adjacent to the blastoderm.
- Morula. 7 days post-fertilization (DPF, *ca.* 9.2 degree-days, DD; Fig. 12b).
- Blastula. 10 DPF (*ca.* 12.2 DD). Epibolic invagination commenced. Also the embryonic disc elongates. Central region of blastoderm raised from the yolk. Blastocoel faint, but visible under illumination.
- Early gastrulation. 14 DPF (*ca.* 14.1 DD). Fifty percent of epibolic invagination. Blastoderm (ectoderm) spread over the yolk surface to encapsulate the endoderm. Embryonic shield and germ ring visible. Neutral keel visible in the centre of the embryonic shield at the animal pole (Fig. 12c).
- Gastrulation. 26 DPF (*ca.* 20.6 DD). Neuralization commenced. Formation of embryonic axis and neural keel or plate (Fig. 12d). The embryo covers one-third of yolk circumference.
- Chephalization. 36 DPF (*ca.* 25.5 DD). Cephalic (Fig. 12e) and caudal (Fig. 12f) regions distinguishable. Embryo covers two-thirds of yolk circumference.
- Organogenesis. 71 PDF (*ca.* 53.8 DD). (Fig. 12g and h). Otic vesicles discernible (Fig. 12i). Pectoral fin buds visible (Fig. 12j). Median and pectoral fin folds evident. Vent fin fold evident. Melanophores present, but sparse along the dorsal surface of the embryo.
- Segmentation. 86 PDF (*ca.* 71.0 DD). Tail-bud stage reached. Somites visible between cephalic and caudal regions. Choroid fissure visible. Rudimentary mouth visible (Fig. 12k). Gut visible, but individual organs indistinguishable. Bilateral rows of melanophores along dorsal surface extended posteriorly from pectoral fin buds to caudal region. Single row of melanophores present along ventral surface. Gut pigmented, particularly in proximity to the vent. Yolk sac pigmented with stellate melanophores evenly spaced over the entire surface.
- Pharyngula. 102 PDF (*ca.* 101.0 DD). Segmentation completed. Tail-free stage reached (Fig. 12l). Embryo pigmentation concentrated along dorsal and ventral surfaces. Melanophores evident on median fin fold (ventral surface), posterior of vent. Dorsal pigmentation visible on head, localized between eyes.
- 116 DPF (*ca.* 143.4 DD). Ventral and dorsal pigmentation progressively darker. Melanophores visible on snout.

- 120 DPF (*ca.* 159.9 DD). Two bilateral of dorsal pigmentation extend posteriorly from head to caudal region.
- 130 DPF (*ca.* 210.2 DD). Two distinct, dense aggregations of melanophores visible on dorsal surface of head. Opercule and preopercule distinguishable. Pectoral fin buds well-developed.

HATCHING AND EARLY REARING

Stages of development and health risks

First hatch can be expected at 120 - 130 degree-days (DD), typically the first week of April at Mersey. At hatch, larvae exit the egg backwards and some were unable to free their yolk-sac and / or head. Once hatched, larvae immediately swam-up in the water column for five to ten minutes, and then sank and rested on the tank floor. For this reason, eggs were transferred to the hatching tank two weeks before hatch (*ca.* 90 DD). A useful method was to set up the Heath tray floating just below the surface of the hatching tank allowing the larvae to swim out of the tray (Fig. 13a). A second method tried once was to transfer 1,000 eggs to MacDonald jars allowing the larvae to exit the jar into early rearing tank via an effluent pipe. Separating the larvae from the unhatched eggs is important, because hatching of a cohort can extend from 7 to 10 days. A third method, not tried at Mersey, but used successfully in Ontario with Lake Whitefish, was a wooden hatching device, similar to a Heath tray is set up floating in the hatching tank and the larvae swim out through mesh side panels (Fig. 13b).

Yolk sac larvae (Fig. 14a) total length (TL, mean $\pm$ SD) was 12.4 $\pm$ 0.86 mm (Hasselman et al. 2007), smaller than rainbow trout (12 to 20 mm TL), and Atlantic salmon (15 to 25 mm TL; Lavens and Sorgeloos 1996). The yolk sac is relatively large (9.97 mm^3) but is absorbed in about four days (Table 4). First feeding is at two days post hatch (dph). The larvae can be fed newly hatched *Artemia*. This is another reason why it was important to segregate unhatched eggs and yolk sac larvae, since *Artemia* debris can get mixed up with the eggs causing fungus problems.

Stocking density at first feeding was 12 larvae/L, following Lake Whitefish protocols. Larvae are very active and swim constantly, usually in the top 2.5 cm of the water column. This behaviour makes it easy to siphon the bottom of the tank to remove *Artemia* debris and faeces.

Rearing environment

Larvae were reared in Mersey River freshwater in a flow-through system (Fig. 15). However, Atlantic Whitefish can be reared in seawater (30 ppt), from pre-metamorphosed larva to adults (Cook and Bentzen 2009). A suitable rearing tank shape was a circular tank, 60 cm diameter with a conical bottom. The conical bottom

resulted in debris settling at the base, making it easy to siphon clean. The water inflow was via a ¾ inch pipe set up at an angle to circulate the water the tank. The flow was *ca.* 4.6 L/min, but was adjusted depending on the behavior of the larvae. Photoperiod tried to simulate the natural light cycle, using a combination of natural light coming through the windows and incandescent clear bulbs. The photoperiod from light bulbs was controlled by timers and adjusted continuously throughout larval stage, but following metamorphosis the photoperiod was not strictly controlled. For example, lights in the main lab (Fig. 15) were manually switched on at the start of the working day and switched off at the end of the working time supplementing any natural light radiating through the windows. In winter this would have extended the natural photoperiod. Late-hatching eggs were always held in a darkened section of the tank. At hatch, temperature was 6 to 8 °C ready for first feeding.

The coloration pattern of larvae differed between families, some had distinctive dark bands along dorsal and ventral sides, and others were very light, almost transparent. Upwelling tanks were used to investigate the effect of light intensity on pigmentation of larvae (three larvae per tank). In brightly illuminated tanks (60 W bulb) larvae lost their dark pigmentation within about 20 minutes, becoming almost transparent. By contrast, in dimly lit tanks the larvae retained their pigmentation.

First feeding (*Artemia salina*)

Atlantic Whitefish larvae were fed newly hatched *Artemia* nauplii from 2 dph to about 30 dph. *Artemia* cysts were hatched in seawater; consequently when nauplii were introduced into the first feeding tank containing freshwater they died within a few minutes due to the osmotic shock. For this reason it was necessary to offer *Artemia* to the larvae at least eight times per day to insure they received sufficient food. However, starvation was never an issue since the larvae were strong feeders. At this stage, larvae are transparent, so it was simple to check if they were eating by looking for 'orange bellies', indicating the presence of *Artemia* in their gut.

Artemia cysts are available from many aquaculture supply companies. *Artemia* can be hatched and cultured at a density of about 3 to 5 g cysts per litre of water. *Artemia* were hydrated in groundwater for about one hour, and then decapsulated for 15 min in a solution of sodium hypochlorite (Commercial quality, Javex®) and sodium hydroxide (NaOH). Then *Artemia* were rinsed with plenty of tap water until there was only a faint smell of chlorine and vinegar was added to neutralise the NaOH. *Artemia* were cultured in artificial seawater, mixing groundwater and Instant Ocean® sea salt to achieve a salinity of 33 ppt. Incubation cones were used, with a vigorous aeration to maintain the cysts and nauplii in suspension, and keep the oxygen level >2 mg/L. *Artemia* should be hatched under continuous, high light intensities (> 1000 - 2000 lux). Incubation temperature was 26 °C using immersion aquarium heaters and setting up the incubators in a cabinet insulated with Styrofoam™. At 26 °C, *Artemia* hatch in *ca.* 24 h. Stage I

nauplii measure between 430 to 520 µm, a suitable size for Atlantic Whitefish larvae. *Artemia* were harvested and rinsed with freshwater to wash off *Vibrio* and then stored in clean seawater in open petri dishes at 4 °C in a fridge. Each petri dish was a meal given to the larvae every hour during the working day.

Weaning to pelleted diet

Once all the larvae were eating *Artemia*, dry feed began to be offered. Initially, very small amounts of dry food were dispensed and the feeding response was carefully monitored. Gradually, over three weeks the proportion of dry feed was increased and the *Artemia* decreased. During the first few years of rearing trials Biokyowa-B was offered initially with Corey Hi-Pro 0.5 mm crumble gradually incorporated until the switch was made to 100 % Corey. However, once Biokyowa-B became unavailable in Canada, Corey was used, without difficulty, to transition larvae from Artemia to prepared food. Larvae eat actively all the time; however, feed rates relative to body size and rearing temperature were not defined at Mersey. As the larvae grew, they occupied more of the water column, but generally preferred to be close to the surface. Salinity tolerance tests indicated the larvae preferred seawater (Cook et al. 2010), but they were reared in freshwater without difficulty. Lake Whitefish cultured in Ontario are offered Otohime dry diet at first feeding and currently are not fed *Artemia*. However, their feeding tactics will have to change since importation of Otohime to Canada stopped in 2013.

A positive phototactic behaviour was clearly evident among larvae at Mersey. If a section of the tank was covered, the larvae quickly swam to the illuminated zone. Compared to first-feeding Atlantic salmon, Atlantic Whitefish larvae showed stronger schooling behavior and were more sensitive to changes in light intensity.

JUVENILES

Rearing conditions and health risks

Metamorphosis from larva to juvenile is complete around 50 dph when the fish are around 40 mm long (Table 5).

Following the transition from larva to juvenile stage (Fig. 16), in June, fish were transferred from early rearing units to larger indoor circular tanks, either 2.0 or 2.6 m diameter. Stocking density was 8 g fish per litre. The fish were moved in water in plastic containers to minimize handling stress. Following transfer, the fish were given a prophylactic salt water bath (20 ppt) treatment for 45 min by dropping the water level and broadcasting the brine to minimize hot spots. As the fish grew, it was necessary to reduce the stocking density by transferring fish to other tanks. Movement of juveniles invariably resulted in mortalities, no matter how careful the transfer. Typically, fungus appeared along the flanks, then redness followed by lesions and death. Salt baths helped reduce the incidence of fungal infections after any disturbance. Tank cleaning

through the first summer was accomplished mainly by careful siphoning. Standpipes were pulled every two days to clean the sumps.

To reduce stress and improve survival among underyearlings, the rearing temperature during the summer was held under 16.5 °C by using chillers or by utilizing inflow water from below the thermocline at the base of the dam. By comparison, fish 1^+ year-old and older were more resistant to high temperature and ate well at 24 °C, or even as high as 27 °C, but mortality did increase at the summer peak temperatures. The best temperature to rear Atlantic Whitefish is unclear. Using a physiological model, a range between 15.5 and 19.1 °C was determined to be optimal (Cook et al. 2010; Cook 2012). The authors considered different pH values, but did not indicate the body size of the fish. Atlantic Whitefish were more active than Atlantic salmon in adjacent tanks, and their oxygen demand was always higher than salmon at a similar body size and stocking density. The oxygen saturation level in the rearing tanks was normally between 90 and 99 %. Occasionally the oxygen decreased to 60 % saturation, but the fish fed and behaved normally.

Juveniles (1^+ year-old) grew well in outdoor square (Swede-style) concrete ponds, either 7.6 or 11 m wide, at a mean stocking density of about 3 kg/m^3. Juvenile Atlantic Whitefish tended to swim close to the water surface, making them easy prey for birds, so anti-predator nets were required. Temperature control of the water in the outdoor tanks was limited, achieved by mixing the surface uptake with the cooler bottom intake or changing to 100 % from the bottom intake. Consequently temperatures in summer were relatively high and not ideal for under-yearlings. Fin-nipping was an isolated problem for about a week after transfer of one batch of juveniles (7 – 8 cm long) to the outdoor tanks. Cannibalism among Atlantic Whitefish was never observed. Older fish (2^+ year-old) were cultured in both 7.6 and 11 m wide concrete ponds, 7.6 m wide, at a mean stocking density of 4 kg/m^3.

Juvenile Atlantic Whitefish were eager feeders during the summer months, and the domesticated fish would swim towards humans, seeking food. The mouth size of Atlantic Whitefish is smaller than salmon and trout of the same body size, requiring a smaller pellet. Feed charts were not developed. Ewos Vita pellets are available in six sizes from 1.5 to 9.0 mm. Appetite decreased in the fall and was minimal over winter. However, the fish continued to take feed throughout winter, albeit rather slowly. Schooling behavior was strong among under-yearlings, but decreased as the fish got bigger. Adults formed loosely aggregated groups. A skeletal deformity became evident among 30 – 40 % of F1 fish by the time they were 2^+ year-old. The deformity was in the caudal peduncle region causing a 'kinked tail'. Starting at 5^+ year old, captive-bred fish showed signs of aging that was not evident among wild fish (the original broodstock), the most prominent being a "bulging" of the head (Fig. 17). The sex ratio of F1 fish was 1:1 based on dissection of many fish. The liver of healthy fish was pale pink.

Growth rates

Atlantic Whitefish grew well in captivity at Mersey, exhibiting a strong seasonal cycle of food intake, with most of their somatic growth restricted to the short summer. For example, between May and October 2007 the mean body weight and fork length of 3^+ year-old fish increased from 97 to 129 g and 21 to 23 cm, an increase of 33 and 10 %, respectively. A year later (Nov. 2008), the same cohort had a mean body weight of 213 g and a mean fork length of 27 cm. Regular assessments of growth were not attempted because of the high sensitivity of the fish to handling stress, particularly when the temperature was >16 °C. By pooling data from several year-classes, a perspective on growth was assembled. Figure 18 provides a useful composite of the change in body size with respect to age. The data also illustrates the considerable variation in body size within each cohort. Body weight of 1^+ year-old fish ranged from 43 to 128 g, and 4^+ year-olds ranged from 151 to 331 g (Fig. 18 left panel). Female Atlantic Whitefish tended to be larger than males, but there was considerable overlap. Among European whitefish, females grew faster than males to age 2^+ year old. Among adults, both sexes had a similar body length but females were heavier, giving them a higher condition factor (Szczepkowski et al. 2010).

The body size of Atlantic Whitefish in during the autumn months was a poor indicator of state of sexual maturation (Table 6). Among mature females, the range in gonadosomatic index (GSI) was similar to European whitefish in November, but the latter reached a GSI of 24 % at full maturity (Rösh 2000). Atlantic Whitefish are iteroparous. Individuals of both sexes at MBF completed sexual maturation for several years in succession. Condition Factor was slightly higher among mature females than immature females, but among males immature fish had a higher condition factor (Table 6). Sexual maturation was completed by the end of November at the earliest, but there was considerable variation between cohorts. For example, in 2008, males age 4^+ year-old matured later in the winter than 3^+ year-old males, but also had a lower Condition Factor than the young fish.

INTRODUCTIONS INTO ANDERSON LAKE AND THE PETITE RIVIÈRE

The Recovery Strategy specified an extension of the geographic range of Atlantic Whitefish beyond their present distribution within the three lakes of the Petite Rivière system. The discovery in 2013 of Chain Pickerel (*Esox niger*) in their natural habitat reemphasized the importance of this initiative (Themelis et al. 2014). To try and identify a suitable habitat, the available chemical, biological and geographical information of 107 lakes in Lunenburg County were analyzed (Wessel 2006). Four potential sites were identified: Hollahan, New Germany, Oakland and Seven Mile Lakes. However, no introductions took place in these lakes because their suitability was too uncertain. Finally, Anderson Lake, Dartmouth, NS was selected as a trial site (see Bradford et al.

2015) because its habitat and water quality appeared suitable for Atlantic Whitefish and human access is limited, all of the adjacent land is owned either by a single private land-owner or the Department of National Defence who controls vehicle access to and from the lake. F1 fish were stocked into Anderson Lake between 2005 and 2012 (Table 7).

The success of the Anderson Lake trial is unclear. Monitoring in 2007 and 2008 indicated fish had survived and some had grown, but others were in poor condition (COSEWIC 2010). Beginning in 2009, ripe males and females have been observed, but there has been no evidence of offspring (Bradford et al. 2015).

Restocking of the Petite Rivière system with F1 fish was confined to downstream of Hebb Lake Dam, the lowest impassable dam on the river, allowing migration to sea (Fig. 19). Restocking of the lakes with F1 fish was not attempted because this action may have further weakened the natural population due to the very restricted genetic variability of the F1 fish coupled with their domestication.

Between May 2007 and March 2009 a total of 12,025 marked F1 fish were released downstream of Hebb Lake Dam (Table 8). Of these, only 1 was were recaptured in November 2012[1] at the Hebb Lake Dam Fish Passage Facility Trap, a 33.5 cm FL and 450 g specimen at the time of capture (Cross 2012). No other re-captures were reported by Bluenose Coastal Action Foundation (Wessel 2006; Page 2010; Cross 2011). Marking the fish released in the Petite Rivière was very important to determine if the re-captured were F1 fish or wild fish. Most (87 %) of the fish released in the Petite Rivière between 2007 and 2009 were adipose fin-clipped. Disappointingly, it was subsequently discovered that after six months fish in culture at Mersey the adipose fin started to regenerate and by 2.5 years post-clipping they were almost 100 % regenerated (although the shape of regenerated fins differed slightly from unclipped fins). Hence a second marking method was employed. Visible Implant Elastomer (VIE) was successfully injected into the pectoral and / or ventral fins of 12 % of the fish released. Fish implanted with VIE were given a minimum of three weeks recovery and associated salt baths before release. Acoustic tags were successfully implanted into 55 fish, but the high cost and limited battery life precluded mass marking.

[1] The fishway became operational on September 24, 2012.

FUTURE RESEARCH

Listed below are ideas for future research on Atlantic Whitefish:

a. Water quality

Factor	Research topic	Reference
Water source	Lakes have less temperature variation than rivers, also hypolimnion helps to have cold water during summer seasons, reducing cost of temperature control	Harris & Hulsman (1991) Gillet (1991)
Aluminum	Toxicity at low pH to eggs and larvae	Keinänen et al. (2003)

b. Broodstock

Factor	Issue	Reference
Age determination	The most reliable method for ageing is otolith, the second scales and then fin rays	Skurdal et al. (1985) Herbest & Marden (2011)
Photoperiod management	Long photoperiod in Fall delays egg production and increases fecundity, but decreases egg size	Gillet (1991)
Light intensity	In European whitefish 150 lux was suitable	Gillet (1991)
Feed ration	It influences sexual maturation	Szczepkowski et al. (2010)
Time of feeding	1+ and 2+ year old fish consume food throughout the day. 3+ and 4+ fish prefer to eat before dusk and after dawn	Szczepkowski et al. (2010)
Stocking density	For European whitefish broodstock 13 kg/m^2 in 2 m^2 tanks is recommended	Szczepkowski et al. (2010)
Determination sexual maturation	Analysis of sex steroids in blood	Rinchard et al. (2001)
Sexual maturation	Size of first maturation depends on pre-reproductive growth rate. Age is more important than body size	Beauchamp et al. (2004)
Milt dilution	It increases the possibility of contact with eggs and extends the motility time	Gilled (1991) Lahnsteiner (2005)
Milt volume	Milt has been stored on crushed ice for 24 h or at 5 °C for 5 h with no reduction in viability. Storing sperm this way allows milt to be collected from several males before fertilizing eggs	Keinänen et al. (2003) Cingi et al. (2010)
Pool milt from several males	Due to low milt production, it is a common practice in whitefish culture in Europe	Lahnsteiner (2005) Ciereszko et al. (2013)
Extender ratio and	It is better to increase milt concentration to	Ciereszko et al.

Factor	Issue	Reference
composition	reduce number of straw and storage space	(2013)
Cryopreservation / Milt quality	Analysis of microsatellites of DNA is a better predictor of cryopreserved milt quality than analysis of fresh milt	Nynca et al. (2012) Fopp-Bayat & Ciereszko (2012)
Determination of milt quality	Fork length a good predictor of sperm swimming speed and body weight a good predictor of testes mass	Blukacz et al. (2010)
Determination of egg quality	Egg carbohydrate composition is a good indicator of egg viability	Lahnsteiner (2005)
Egg characterization	Egg size from wild fish differs from reared fish. Also there are differences in the egg size depending on the time of spawning	Gillet (1991)

c. Egg incubation

Factor	Issue	Reference
Sticky eggs	Several methods have been used to removal the adhesiveness from eggs of different species. The selection should be consider time needed to remove adhesiveness, survival rate, quality of larvae (deformities), cost of material and labour	Smigielski & Arnold (1972) Krise (1993) Rottmann et al. (1988) Isaac Jr. & Fries (1991) Ringle et al. (1992)
Temperature	It affects the incubation duration. Temperatures highest that at Mersey has been used (up to 6.4 °C). But >7 °C, impairs fertilization and increases incidence of deformities among embryos (eggs)	Drouin et al. (1986) Harris & Hulsman (1991) Hooper (2006) Cingi et al. (2010)
Incubator device	Eggs incubated in jars hatched sooner and better synchronized than ones incubated in trays	Bidgood (1974)
Monitor infections	Non-lethal exposure to *Pseudomonas*, can make embryos more susceptible to subsequent bacterial challenges	von Siebenthal et al. (2009)
Oxygen requirement	The minimum requirement of oxygen concentration varies with species and temperature	Czerkies et al. (2002)
Photoperiod	Lake whitefish eggs are incubated in darkness but European whitefish are incubated with natural photoperiod (unknown intensity)	Harris (1992) Eckmann (2000) Hooper (2006)
Light	It is the main factor that affect embryonic development of whitefish, species adapted to long-term period with low temperatures	Chernyaev (2007)
Fungus treatment	The use of NaCl mixed with $CaCl_2$ has been more effective to treat chinook salmon eggs than only NaCl	Edgell et al. (1993)

d. Hatching and early rearing

Factor	Issue	Reference
Monitor infections	Eggs infected with *Pseudomonas* or only it presence in the rearing water can advance the timing of hatch	Wedekind (2002)
Hatching management	To increase of temperature between 2 to 6 ºC within 24 h above incubation temperature synchronizes hatching within 10 to 12 h. But reducing temperature to delay the hatch increases initial survival	Drouin et al. (1986) Champigneuile & Rojas-Beltran (1990) Hooper (2006)
Larvae characterization	Incubation duration effects larvae size, early hatched larvae are smaller than later hatches, and also have bigger yolk-sac	Bidgood (1974) Champigneulle & Rojas-Beltran (1990)
Photoperiod management	Natural photoperiod was used initially (1986) but then 24 h light has been used since a least 1991	Drouin et al. (1986) Beltran-Rojas & Champigneulle (1991) Hooper (2006)
Light intensity	Early rearing 120 to 170 lux. After weaning 200 to 300 lux	Beltran-Rojas & Champigneulle (1991) Harris & Hulsman (1991) Harris (1992)
Phototactism	Early lake whitefish larvae are photopositive but after week 4 are more photonegative	Häkkinen et al. (2003) Hooper (2006)
Nutrition deficiency	Early larvae need crustacean (*Artemia* or meal) to prevent opercular and / or spinal deformities, probably due to lecithin or phospholipids deficiency	Drouin et al. (1986) Harris & Hulsman (1991)
Artemia supply	Automatic feeder that allows a continuous supply	Drouin et al. (1986)
Development of digestive tract	When the digestive tract is fully developed (0.1 to 0.14 g body weight) weaning onto a dry pellet can start	Drouin et al. (1986) Harris & Hulsman (1991)
Temperature	Rearing water temperature has more influence on growth than feed rate	Drouin et al. (1986)
Feeding time	With 24 h photoperiod, 24 h feeding. Frequency 5 to 15 min and a duration of 0,1 to 0.5 s	Beltran-Rojas & Champigneulle (1991) Harris (1992)
Diseases	Bacterial gill infections are frequent in lake whitefish, when temperature is high and / or oxygen is low. To reduce stress, cleaning must be conducted at the	Harris (1992) Hooper (2006)

Factor	Issue	Reference
	same time every day	
Bacterial gill infections	Salt bath and Chloramine-T bath 5 mg/L, without stopping water flow to prevent stress	Champigneulle & Rojas-Beltran (1990) Hooper (2006)
Tank design	Inflow pipe below the water surface is better than above to prolong the floating time of feed	Enz et al. (2001)
Tank design	At early rearing only a submerged pipe supply water. After four weeks, a second pipe is added to increase self-cleaning	Hooper (2006)

e. Juveniles

Factor	Issue	Reference
Tank design	Lake whitefish grow faster in raceways than in circular tanks, and also size dispersion is lower. Surface to volume ratio. Water depth <1 m is preferred	Harris (1992) Hooper (2006)
Stocking density	Lake whitefish grow better at 30 to 40 g / L	Harris (1992)
Photoperiod management	24 h light	Harris (1992) Siikavuopio et al. (2012)
Light intensity	150 lux but also 1100 to 2697 lux has been used in European whitefish	Szczepkwski et al. (2006) Siikavuopio et al. (2012)
Feeding regimen	To give the same daily meal at different feeding periods does not affect either final feed intake or growth. European whitefish exhibit compensatory growth mechanisms	Koskela et al. (1997) Känkänen & Pirhonen (2009)
Feed rate	The effect of the feed ratios in the growth rate change depending on the duration of the experiment	Wunderlich et al. (2011)
Improvement of growth rate	Constant temperature (10 °C) and 24 h photoperiod throughout the year	Siikavuopio et al. (2012)
Temperature	Preferred temperature varied inversely with juveniles size, 2 to 4 g body weight	Edsall (1999)
Oxygen consumption	Oxygen consumption increased as water temperature increased but not linearly	Szczepkwski et al. (2006)
Phosphorus requirement	European whitefish required lower phosphorous content than salmon. 0.65 vs. 0.8 %	Vielma et al. (2002)
Carbohydrate tolerance	European whitefish do not tolerate more than 10 % carbohydrate	Vielma et al. (2003)

f. Introduction and re-stocking

Factor	Issue	Reference
Fish size	Lake whitefish are re-stocked at 20 to 25 g	Harris (1992) Hooper (2006)
Stocking density for transport	Lake whitefish are transported at 100 g / L	Hooper (2006)

g. Pathogenic diseases

Disease	Comment	Reference
Kidney cnidaria parasite	*Sphaerospora coregoni* has been described in European whitefish	El-Matbouli & Hoffmann (1996)
Vibriosis	*Listonella* (*Vibrio*) *anguillarum* has been described in European whitefish. Vaccines work well	Lönnström et al. (2001)
Furuncolosis	*Aeromonas salmonicida salmonicida* has been described in European whitefish; vaccines work well. Also found in lake whitefish	Lönnström et al. (2001) Loch and Faisal (2010a)
Furuncolosis	Six types of *Aeromonas* has been described in lake whitefish	Loch & Faisal (2010b)
Cyanobacteria	*Planktothrix rubescens* blooms produce physiological stress that increase ectoparasitic infestation in European whitefish	Ernst et al. (2007)
Eye fluke (digenetic parasite)	*Diplostomum spathaceum* has been described in European whitefish. It induces cataracts, when the entire eye is covered, fish lose weight	Karvonen & Seppälä (2008)
Bacterial kidney disease	*Renibacterium salmoninarum* has been described in lake whitefish	Faisal et al. (2010a)
Swimbladder nematode	*Cystidicola farionis* has been described in lake whitefish	Faisal et al. (2010b)

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TABLES

Table 1. Atlantic Whitefish broodstock collection at Hebb and Milipsigate lakes, Petite Rivière 2000 to 2004.

Year	Date	Fishing effort, days	Method	Nº of fish	Comment
2000	Oct. 24 to 30	2	Angling	4	
2000	Nov. 9 to Dec. 6	3	Trapping	4?	Method ceased due to high mortality
2001	Jun. 11 to 14	5	Angling	7	Collection stopped due to water temperature (>16.5 °C)
2001	Nov. 25	1	Seining	1	
2002	May 22 to Jun.8	4+	Angling	33	Not clear the days of capture between May 28 and June 8
2003	Oct. 1o to 27	5	Angling	8	
2003	Oct. 31	1	Trapping net	8	Milipsigate Lake. Not clear if more captures after Oct 31
2004	Oct.	1	Angling	0	
2004	Oct.	1	Seining	0	

Table 2. Mortalities of Atlantic Whitefish broodstock at Mersey Biodiversity Facility. 2003 to 2008.

Date	Weight, g	Fork length, cm	Time in captivity, years	Comments
August 9, 2003	159	24.5		
January 24, 2004	1000	41.0		Lesion caudal peduncle (spawning)
March 27. 2004		36.5		Lesion caudal peduncle (spawning)
April 11, 2004	890	42.0		Lesion caudal peduncle (spawning)
April 23, 2004	675	37.0		Lesion caudal peduncle (spawning)
May 1, 2004	845	40.5		Lesion caudal peduncle (spawning)
January 18. 2005	1340	43.5		Blockage / rough spawning
July 20, 2005	553	36	2	Not eating – slink
July 30, 2005	414	31.5	2	
August 19, 2005	209	25.3	2	F1
August 19, 2005	1450	47.0	4	
August 23, 2005	214	25.4	2	F1
October, 8 2005	1442	46.2	4	
October 10, 2005	1940	52.0	4	Lesion caudal peduncle
November 30, 2005	340	31.6	2	Spawning related
November 30, 2005	580	36.4	2	Spawning related
December 9, 2005	580	33.9	2	Spawning related
December 13, 2005	760	40.0	2	Spawning related
December 27, 2005	1080	41.0	3	Blind / eroded caudal
December 30. 2005			3	Recently spawned
December 30. 2005			3	Recently spawned
January 1, 2006	560	37.6	3	
January 5, 2006	1220	39.7	4	Died prior to spawning
January 23, 2006	2100		5	Spawning related
March 4, 2006	460	34.0	3	F1
March 27, 2006	600		3	F1. Fungus growth in head region
April 3, 2006	620	37.0	3	F1
May 2, 2006	540	37	3	F1. Broken vertebrae
May 24, 2006	620	36.8	3	F1
July 4, 2006	1780	46.6	4	
August 2, 2006	750	37.0	3	F1. Lesion in head region
January 3, 2007	943	43.5	4	F1. From film tank
January 23, 2006	880	39.2	4	F1. Still holding eggs. Female3
April 9, 2007	1411	45.0	5	Fungus on caudal peduncle
June 4, 2007	460	36.2	4	F1
July 20, 2007	2340	51.5	6	Injury in head/nose region
January 23, 2008	1440	44.1		Still holding eggs. Female
February 26, 2008	1399	45.0		

Table 3. Broodstock Atlantic Whitefish spawned at Mersey Biodiversity Facility. Body sizes show the mean and the range (in brackets).

	Females			Males		
Spawning season	**n**	**Fork length, cm**	**Body weight, g**	**n**	**Fork length, cm**	**Body weight, g**
Dec. 2001 - Jan. 2002	3	30.8 (29.5 to 32.0)	311 (200 to 370)	9	32.2 (27.5 to 35.0)	406 (198 to 558)
2002/03	14	32.5 (29.5 to 35.0)	475 (316 to 638)	18	34.8 (31.0 to 40.5)	555 (363 to 970)
Dec. 2003	14	38.4 (35.5 to 41.2)	916 (660 to 1595)	14	40.2 (35.5 to 43.5)	922 (720 to 1100)
Nov. 2004 - Jan. 2005	9	38.3 (30.0 to 43.5)	829 (387 to 1340)	13	36.6 (21.0 to 46.5)	704 (240to 1308)
Nov. 2005 - Jan. 2006	13	37.0 (31.0 to 47.0)	815 (380 to 1580)	16	38.7 (31.0 to 49.0)	862 (320 to 2100)

Table 4. Atlantic Whitefish larval stages of development as rearing temperatures increased from 2.6 °C, at hatch to 12.5 °C, at 50 days post hatch (dph) a total of 472 degree-days (Hasselman 2003).

Age, dph	**Total length, mm**	**Weight, mg**	**Comments**
0	11.26	17.2	Yolk sac larva (n = 1). Yolk sac volume 9.97 mm^3 (Fig. 6.2a)
9	11.6	14	Yolk sac larva (n = 1). Yolk sac volume 5.18 mm^3. Exogenous feeding was observed by 4 dph
17	15.2 to 16.0	21 to 23	Yolk sac larvae (n = 3). Yolk sac 0.29 to 0.44 mm^3, almost completely absorbed
23	18.4 to 19.0	34 to 43	Pre-flexion larvae (n = 2). Yolk sac 0.18 to 0.31 mm^3 (Fig. 6.2b)
30	21.0 to 22.0	58 to 63	Pre-flexion larvae (n = 2)
36	17.0 to 26.1	129 to 131	Post-flexion larvae (n = 3, Fig. 6.2c)
43	32.4 to 32.7	251 to 287	Post-flexion larvae (n = 2). Lateral line fully developed
50	41.1	647	Post-flexion larva (n = 1). Scales forming

Table 5. Atlantic Whitefish early juvenile stages of development (Hasselman 2003).

Age, days post hatch	Total Length, mm	Body weight, g	Comments
50	41.1	0.61	Metamorphosed larva (n = 1). Fully scaled (Fig. 7.1, upper panel)
57	49.0 to 58.0	0.97 to 1.81	Juveniles (n = 6, Fig. 7.1, lower panel)
64	58.6 to 66.0	2.21 to 3.19	Juveniles (n = 2)

Table 6. Mean (range) gonadosomatic index (GSI), fork length (FL) and condition factor (CF) of sexually immature (I) and mature (M) Atlantic Whitefish cultured at Mersey Biodiversity Facility. N = number of fish.

		Males				Females			
Date/ age	Matu-rity	GSI	FL, cm	CF	N	GSI	FL, cm	CF	N
Fall 2006 / 3+	I	2.6 (2.0 - 3.3)	28.6 (25 - 31)	1.1 (0.9 - 1.2)	21	0.6 (0.5 - 0.8)	28.2 (26 – 31)	1.1 (0.9 - 1.2)	12
	M	-	-	-	-	9.8 (8.0 – 11.5)	28.2 (26 – 30)	1.1 (1.1 - 1.2)	17
Nov. 20, 2008 / 3+	I	1.5 (0.6 - 2.1)	25.4 (22 - 27)	1.3 (1.0 - 1.9)	7	1.4 (0.6 - 2.1)	27.3 (25 – 29)	1.0 (1.0 - 1.1)	10
	M	14.0 (11.6 – 15.2)	26.8 (26 – 28)	1.2 (1.1 - 1.2)	4	12.9 (9.4 – 16.7)	26.8 (25 – 28)	1.1 (1.0 - 1.2)	9
Nov. 20, 2008 / 4+	I	1.2 (0.1 - 2.5)	29.9 (27 - 32)	0.9 (0.7 - 1.0)	13	1.1 (0.2 – 2.0)	29.9 (29 – 32)	0.9 (0.7 – 1.0)	8
	M	-	-	-	-	11.5 (7.8 – 16.5)	30.5 (29 – 32)	1.0 (0.9 - 1.1)	9

Table 7. Juveniles and larvae Atlantic Whitefish introduced into Anderson Lake between 2005 and 2012.

Release data	N	Spawning year	Weight, g	Fork length, cm	Age at release, yr+
Nov. 2005	1500	2003	125	22	1
Apr. 2006	4,000	2005	0.01	1.5	Yolk sac larvae
Apr. 2006	750	2004	50	17	1
Apr. – May 2006	15	2003	150	24	2
Oct. 2006	750	2004	70	19	1
May 2007	3,000	2006	0.01	1.5	Yolk sac larvae
May 2007	750	2005	40	15	1
Oct. 2007	756	2005	50	17	1
Nov. 2008	184	2004	210	26	3
Nov. 2008	212	2003	260	30	4
Nov. 2012	80	2005/2006	300	32	6 / 5

Table 8. Juveniles re-stocked downstream of the lowest dam on the Petite Rivière system between 2007 and 2009. From: Cross (2012).

Release date	N	Spawning year	Body weight, g	Fork length, cm	Age at release, yr+
May 2007	3,015	2004	90	20	2
Oct. – Nov. 2007	5,760	2004	150	24	2
Jun. 2008	1,750	2005	100	21	2
Mar. 2009	1,500	2005	120	23	3

FIGURES

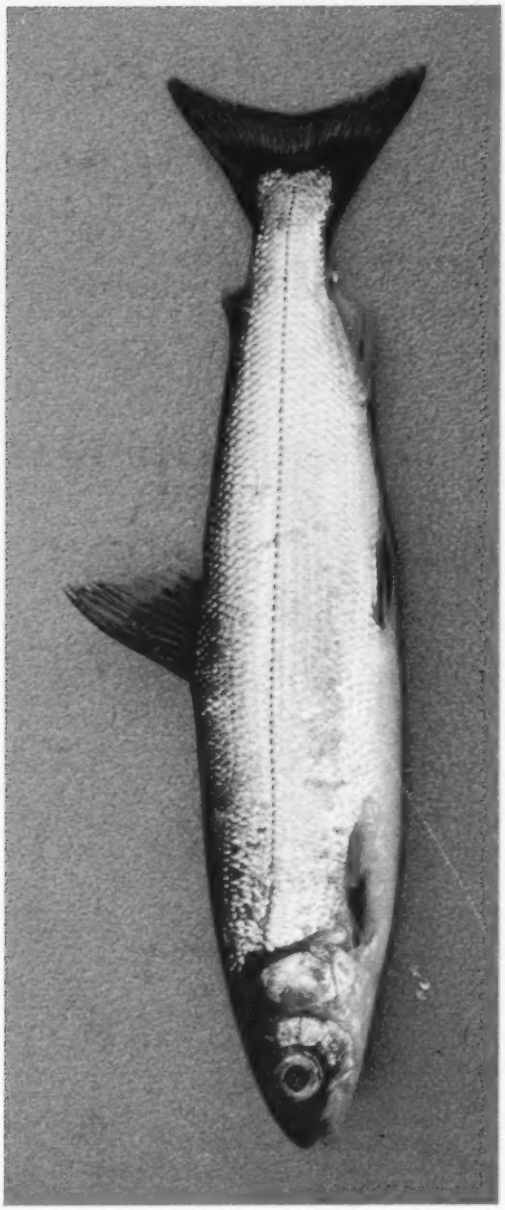

Figure 1. Image of an Atlantic Whitefish reared from the egg stage at Mersey Biodiversity Facility, Milton, NS.

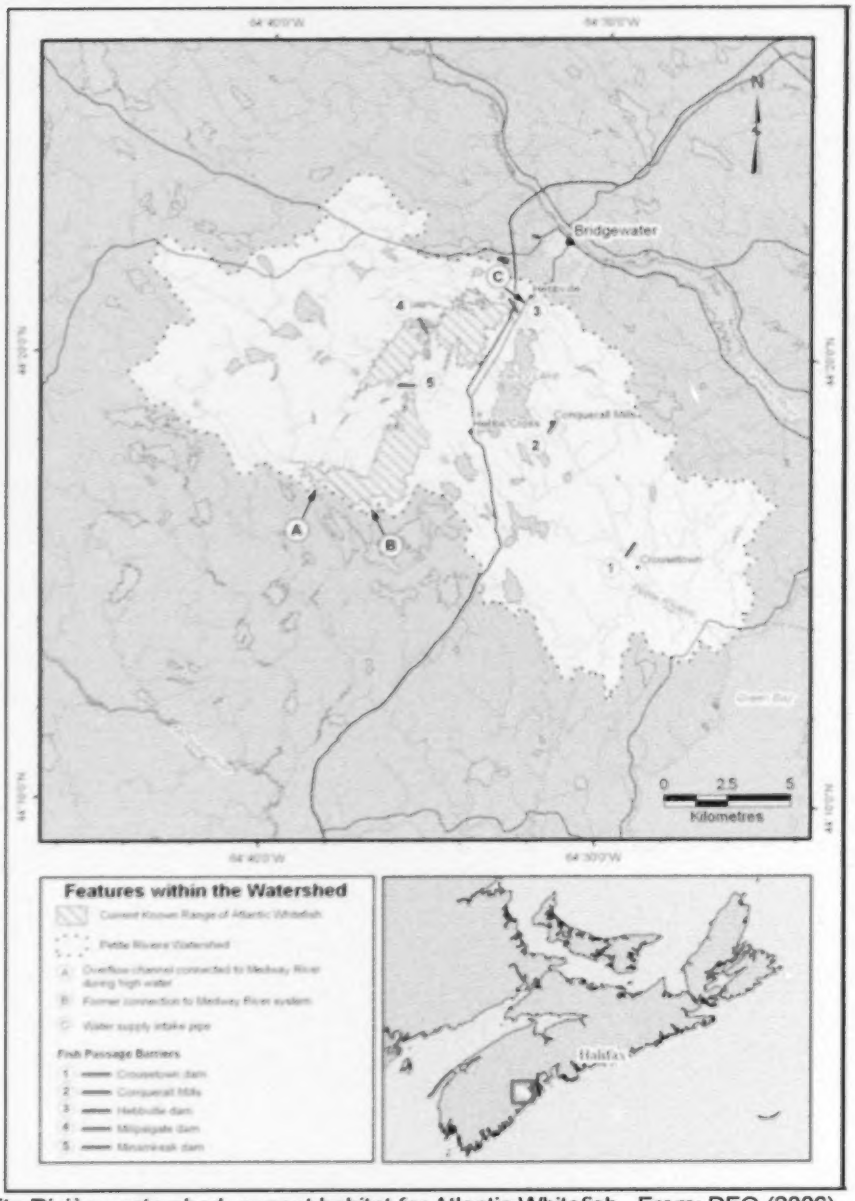

Figure 2. Petite Rivière watershed, current habitat for Atlantic Whitefish. From: DFO (2006).

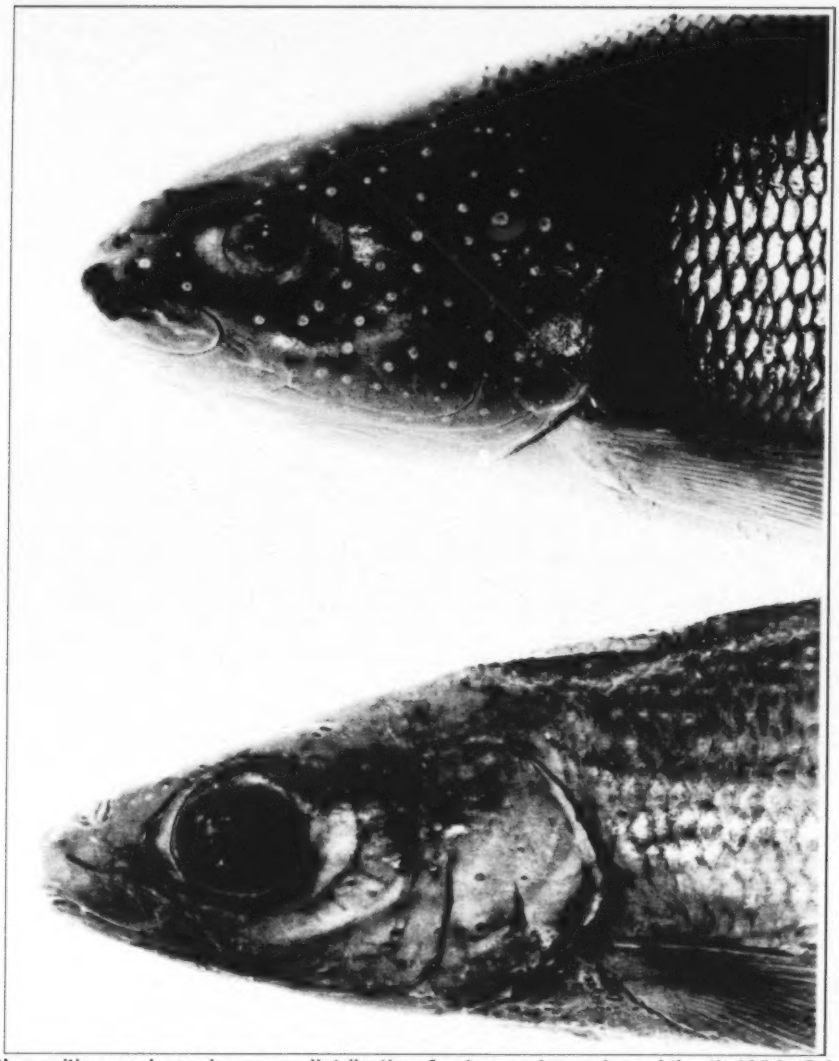

Figure 3. Mouth position and pearl organs distribution for (upper image) an Atlantic Whitefish male with a terminal mouth and larger and numerous pearl organs and (lower image) a lake whitefish male with a sub-terminal mouth and smaller pearl organs. From Edge et al. (1991).

Figure 4. View of the exterior of Mersey Biodiversity Facility, NS. Outdoor ponds for culture Atlantic Whitefish and Atlantic Salmon. Photo courtesy J. Whitelaw, DFO.

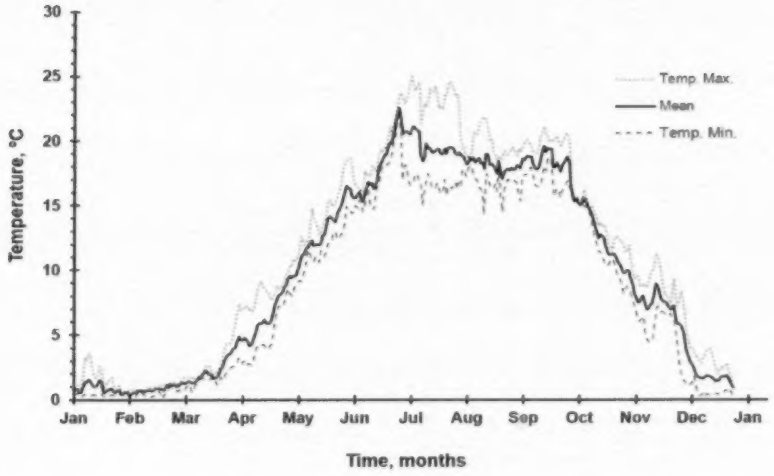

Figure 5. Water temperature (°C) at the Mersey Biodiversity Facility 2003 to 2007. Daily mean from recordings every 30 min (2003-04) or every 4 h (2005-07).

Figure 6. Location for wild Atlantic Whitefish collection, pool below the dam between Hebb and Milipsigate Lakes. Photo courtesy J. Whitelaw, DFO.

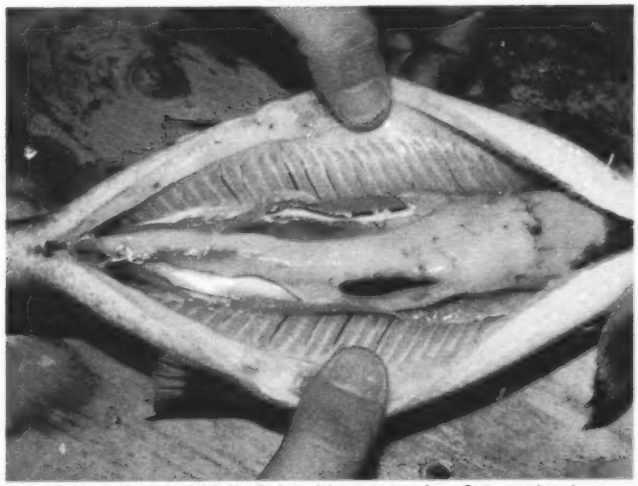

Figure 7. Abdominal cavity of an Atlantic Whitefish with excessive fat content around the organs associated with the fish being fed a salmon diet containing 20 % fat. Photo courtesy J. Whitelaw, DFO.

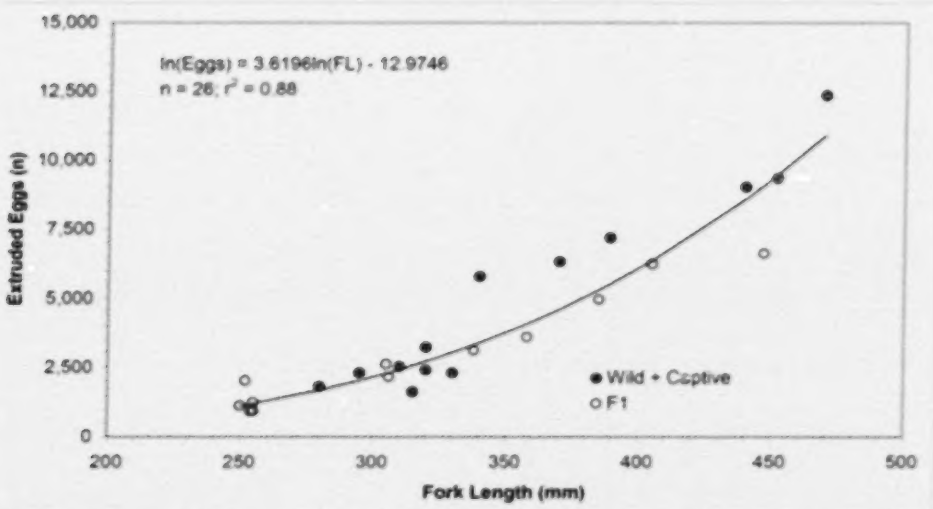

Figure 8. Fecundity as number of extruded eggs (n) versus fork length (mm) of wild female Atlantic Whitefish in captivity (closed circle) and their F1 (clear circle). From: Bradford et al. (2010).

Figure 9. Artificial spawning of Atlantic Whitefish at Mersey Biodiversity Facility. a) Egg collection; b) Eggs in plastic bowl; c) Egg fertilization with milt added with an eye-dropper. Photos courtesy J. Whitelaw, DFO.

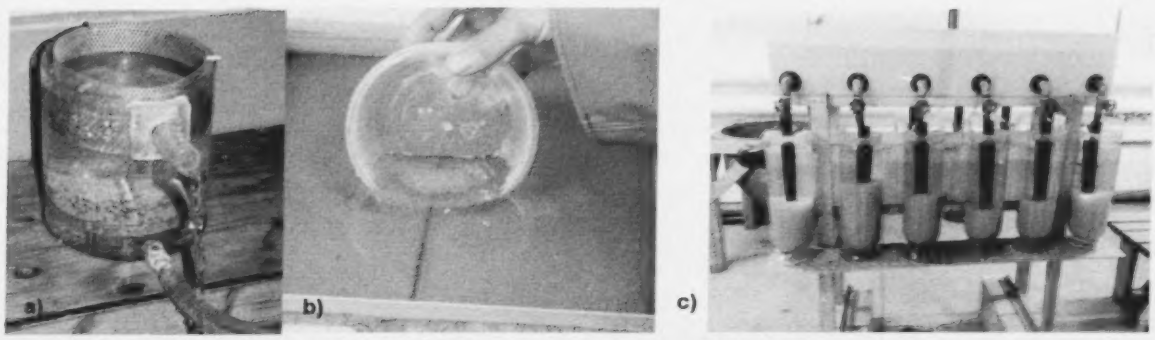

Figure 10. Atlantic Whitefish eggs incubation devices at Mersey Biodiversity Facility. a) 3-litre MacDonald jar; b) Heath tray. Photos by S. O'Neil, courtesy J. Whitelaw, DFO; c) Lake Whitefish eggs incubated in MacDonald jar at White Lake Hatchery, ON. Photo courtesy R. Zheng, MNR, ON.

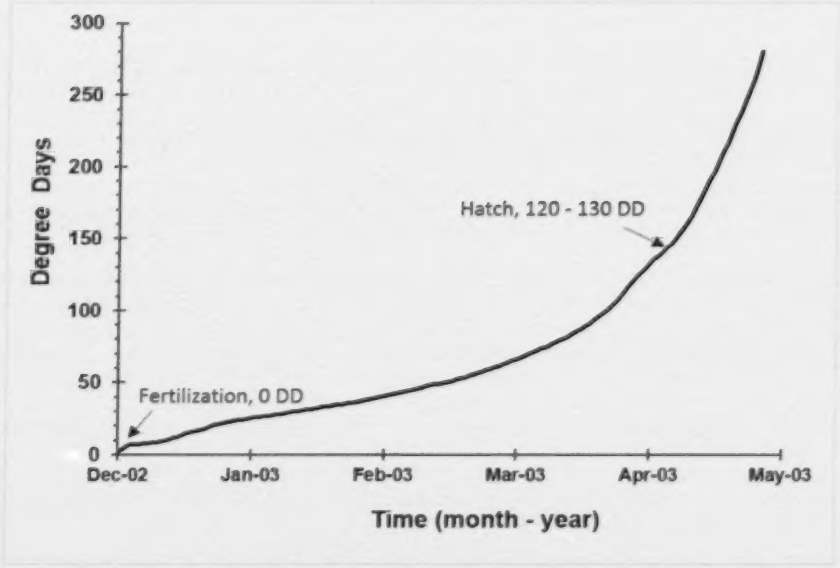

Figure 11. Accumulated degree-days (DD) of Atlantic Whitefish eggs incubated at Mersey Biodiversity Facility.

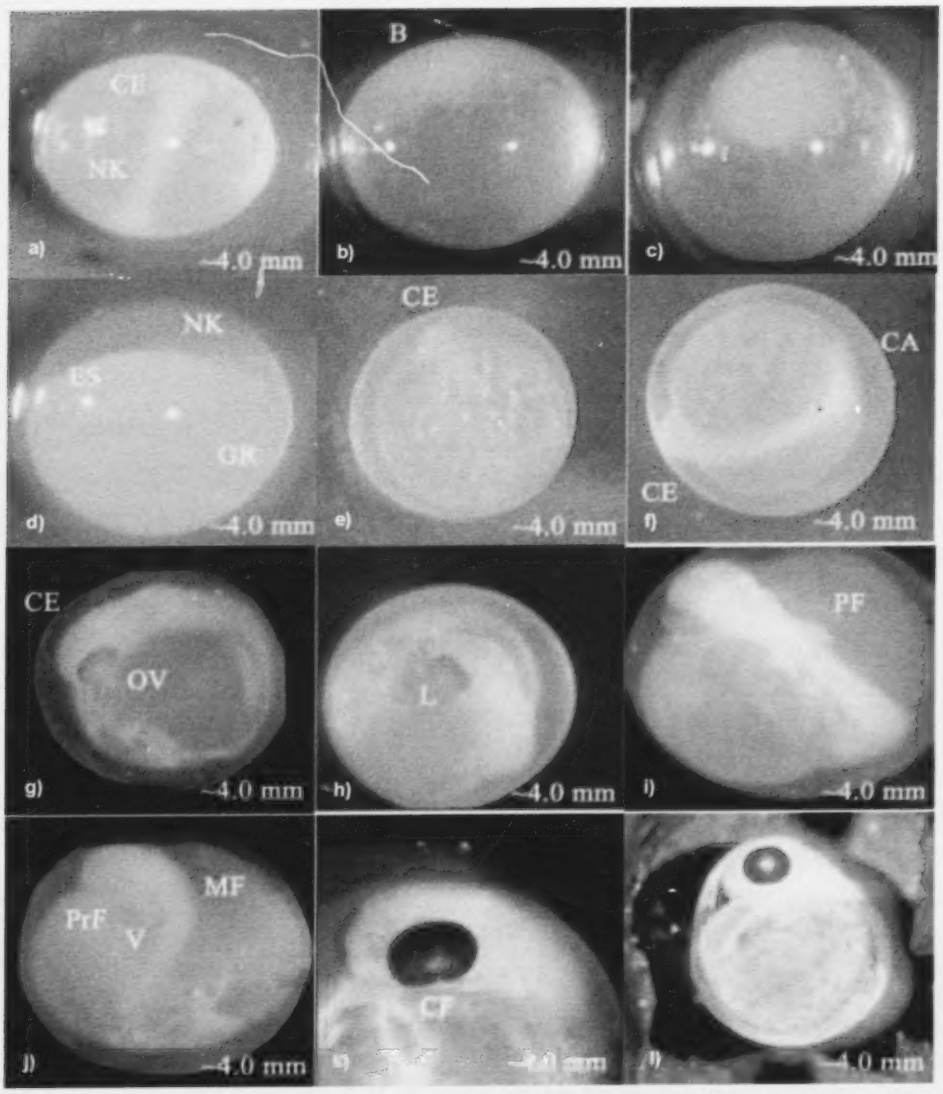

Figure 12. Atlantic Whitefish embryogenesis. a) Fertilized egg – discoblastula, 2.5 h; b) morula, 7 days; c) epibolic invagination and gastrulation, 14 days; d) neutralization, 26 days; (e and f) cephalization, 36 days; g and j) organogenesis, 71 days; k) segmentation, 86 days; l) pharyngula (chorion removed), 102 days. Abbreviations denote morphological features as follows: B, blastomeres; CA, caudal region; CE, cephalic region; CF, choroid fissure; ES, embryonic shield; GR, germ ring; L, lens; MF, median fin fold; NK, neural keel; OV, optic vesicle; PF, pectoral fin bud; PrF, preanal fin fold; V, vent. From: Hasselman et al. (2007).

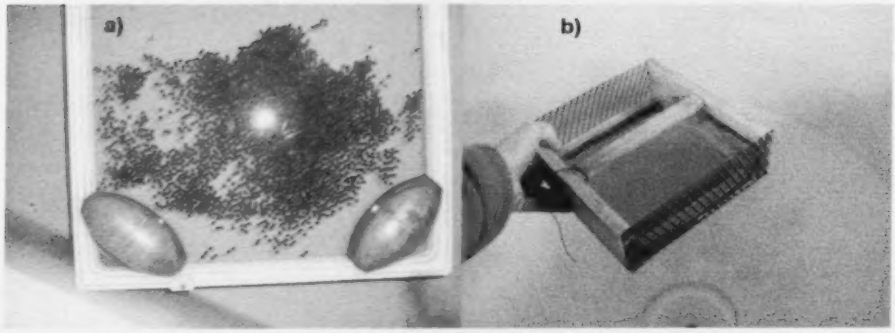

Figure 13. Hatching devices for whitefish. a) Floating Heath tray in rearing tank used to hatch Atlantic Whitefish at Mersey Biodiversity Facility, NS; b) Hatching device used for Lake Whitefish larvae at White Lake Hatchery, ON. Photos courtesy J. Whitelaw, DFO.

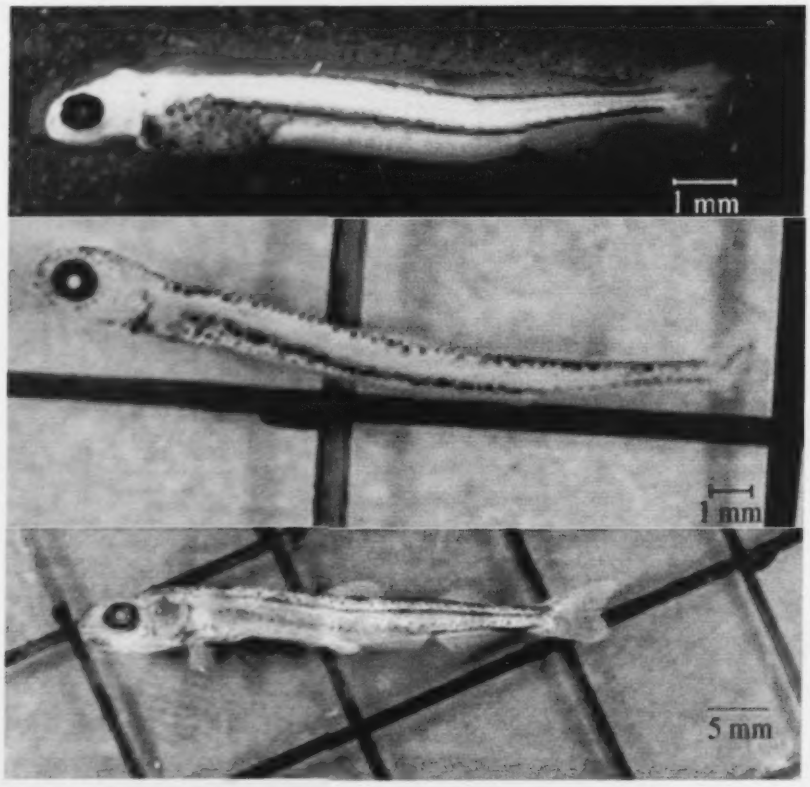

Figure 14. Atlantic Whitefish larvae. a) Yolk-sac larva; b) pre-flexion larva; c) post-flexion larva. From: Hasselman et al. (2007).

Figure 15. Interior of Mersey Biodiversity Facility, NS. From left to right: a) incubation flow-through tanks, b) larvae rearing tanks, c) broodstock tanks. Illumination from both fluorescent tubes and natural light though windows adjacent to broodstock tanks. Photo courtesy J. Whitelaw, DFO.

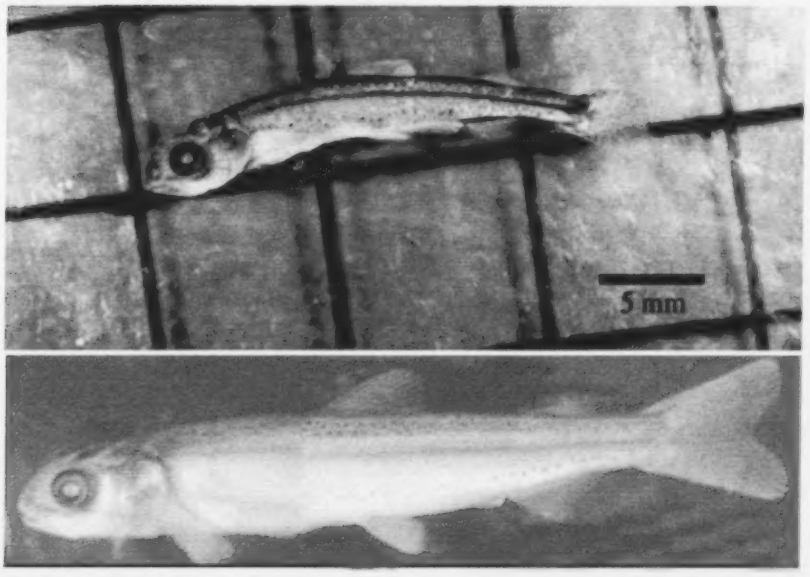

Figure 16. Upper panel: Atlantic Whitefish late larva (from Hasselman et al. 2007). Lower panel: early juvenile (from Hasselman and Bradford, 2012).

Figure 17. "Bulging" head syndrome. Common among Atlantic Whitefish >5 year-old reared at Mersey Biodiversity Facility, possible sign of aging or chronic culture. Photo courtesy J. Whitelaw, DFO.

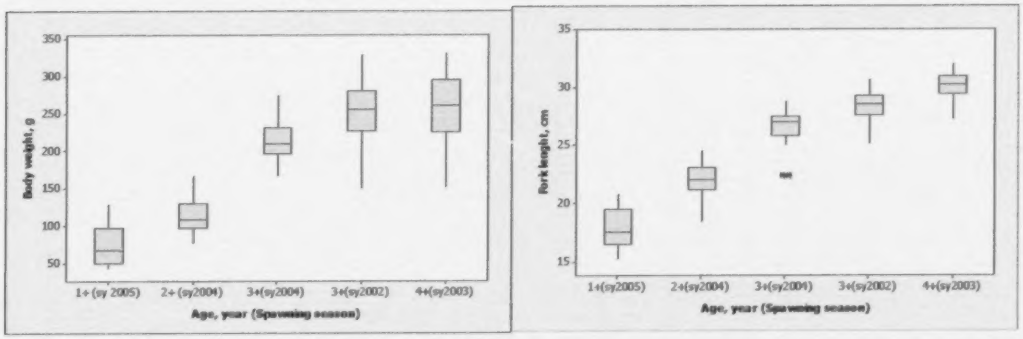

Figure 18. Distribution of body weight (left panel) and fork length (right panel) of four cohorts of Atlantic Whitefish ranging in age from 1+ to 4+ year-old, cultured at the Mersey Biodiversity Facility. The box-plots show the median (2nd quartile), upper and lower quartiles, and the range. The asterisks indicate outliers. 1+(Spawning year, sy2005) and 2+(sy2004) n = 60; 3+(sy2004) and 4+(2003) n = 30; 3+(sy2002) n = 50.

Figure 19. Left panel, position of Petite Rivière watershed in Nova Scotia. Right panel, Atlantic Whitefish release locations in May and Oct. - Nov. 2007. 1) Upstream of Bargain Bob's, 2) Main bridge below Pump Station, 3) Pump Station, 4) New bridge, 5) Main bridge upstream of Pump Station, 6) Behind Elementary School, 7) William's private bridge, 8) Above Crousetown Dam. White scale bar = 1 km.

APPENDIX I

Water quality at Mersey Biodiversity Facility. Raw river water, before, and water used in the hatchery, after limestone treatment. October 2009 and December 2010. Units are mg/L unless indicated.

Calculated Parameters	2009		2010	
	Before	After	Before	After
Anion Sum (me/L)	0.120	0.110	0.120	0.120
Bicarb. Alkalinity (calc. as $CaCO_3$)	<1	<1	<1	<1
Calculated TDS	10	11	11	12
Carb. Alkalinity (calc. as $CaCO_3$)	<1	<1	<1	<1
Cation Sum (me/L)	0.190	0.280	0.230	0.250
Hardness ($CaCO_3$)	3	6	3	5
Ion Balance (% Difference)	22.6	43.6	31.4	35.1
Total Alkalinity (Total as $CaCO_3$)	<5	<5	<5	<5
Dissolved Chloride (Cl)	4	4	4	4
Colour (TCU)	69	75	110	110
Nitrate + Nitrite	<0.05	<0.05	<0.05	<0.05
Nitrate (N)	<0.05	<0.05	<0.05	<0.05
Nitrite (N)	<0.01	<0.01	<0.01	<0.01
Nitrogen (Ammonia Nitrogen)	<0.05	<0.05	<0.05	<0.05
Total Organic Carbon (C)	6.3	6.8	7.4	7.6
Orthophosphate (P)	<0.01	<0.01	<0.01	<0.01
pH	5.86	6.43	5.09	5.45
Reactive Silica (SiO_2)	0.9	0.9	1.8	1.8
Dissolved Sulphate (SO_4)	<2	<2	<2	<2
Turbidity (NTU)	1.0	0.9	1.3	1.6
Conductivity (uS/cm)	25	28	26	26

APPENDIX II

Total heavy metal concentration (µg / L) at Mersey Biodiversity Facility. Raw river water, before, and water used in the hatchery, after limestone treatment. October 2008 and December 2010.

Metals	2009		2010	
	Before	After	Before	After
Aluminum (Al)	140	160	203	211
Antimony (Sb)	<2.0	<2.0	<1.0	<1.0
Arsenic (As)	<2.0	<2.0	<1.0	<1.0
Barium (Ba)	<5.0	<5.0	2.9	3.1
Beryllium (Be)	<2.0	<2.0	<1.0	<1.0
Bismuth (Bi)	<2.0	<2.0	<2.0	<2.0
Boron (B)	<5.0	<5.0	<5.0	<5.0
Cadmium (Cd)	<0.03	<0.03	<0.017	<0.017
Calcium (Ca)	500	1500	721	1260
Chromium (Cr)	<2.0	<2.0	<1.0	<1.0
Cobalt (Co)	<1.0	<1.0	<0.4	<0.4
Copper (Cu)	<2.0	<2.0	<2.0	<2.0
Iron (Fe)	260	290	335	340
Lead (Pb)	<0.5	<0.5	<0.5	<0.5
Magnesium (Mg)	300	500	369	393
Manganese (Mn)	42	44	42.1	41.8
Molybdenum (Mo)	<2.0	<2.0	<2.0	<2.0
Nickel (Ni)	<2.0	<2.0	<2.0	<2.0
Phosphorus (P)	<100	<100	<100	<100
Potassium (K)	300	300	236	255
Selenium (Se)	<2.0	<2.0	<1.0	<1.0
Silver (Ag)	<0.5	<0.5	<0.10	<0.10
Sodium (Na)	2900	3200	3080	3010
Strontium (Sr)	<5	6	5.2	5.4
Thallium (Tl)	<0.1	<0.1	<0.1	<0.1
Tin (Sn)	<2.0	<2.0	<2.0	<2.0
Titanium (Ti)	<2.0	<2.0	<2.0	<2.0
Uranium (U)	<0.1	<0.1	<0.1	<0.1
Vanadium (V)	<2.0	<2.0	<2.0	<2.0
Zinc (Zn)	<5.0	<5.0	<5.0	5.1